

American Journal of Clinical Pathology

S. E. Gould, M.D., *Editor*

Volume 18

Baltimore
The Williams & Wilkins Company
1948

H 8

41924.

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A PHOTO-ELECTRIC OXYHEMOGRAPH

A CONTINUOUS METHOD FOR MEASURING THE OXYGEN SATURATION OF THE BLOOD*

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The demand for a reliable procedure for continuously recording the oxygen saturation of the blood has led to the publication of several photo-electric methods.^{4, 5, 9, 10, 12-16} These methods use the well-known color variation of the blood as an indication of changing oxygen content and provide instrumental means, as a supplement to visual observations, for quantitatively measuring this color change. A more specific statement of the operating principle of these devices is that in the red region of the spectrum (above 650 $m\mu$) reduced hemoglobin absorbs a greater amount of light than oxyhemoglobin, and thus a variation of red light absorption indicates a change in the ratio of oxygenated to reduced hemoglobin.

The design of equipment for this purpose has varied in such features as type of photocell, site of application and method of indication. One of the limiting factors in this earlier work was the inadequacy of amplification methods. Conventional D.C. amplifiers are subject to instability. On the other hand, if no amplification is used, the indicating instrument is delicate and generally unsuited for continuous recording.

It is the purpose of this presentation to describe a bichromatic photo-electric method of measuring blood oxygen saturation, as registered through the ear, a unique feature of the method being the amplification by means of a "contact modulated" D.C. amplifier¹¹ which is both stable and sensitive and allows the making of a permanent record on a rugged D.C. recording milliammeter. The use of this special amplifier was suggested by Mr. C. F. Kettering to Dr. R. D. McClure as a means of improving the amplification applied in our older models.

Although the first photo-electric estimation of oxyhemoglobin is attributed to Nicolai,¹⁵ the fundamental work in blood oximetry was performed by Kramer,^{9, 10} and Matthes^{12, 13} in 1934 and 1935, each working independently. Kramer recorded variations in oxyhemoglobin *in vitro* and *in vivo*, using a method based on the spectral differences of hemoglobin and oxyhemoglobin in the red wave length region (620-770 $m\mu$). He showed that Beer's law of optical absorption may be applied to hemoglobin solutions and hemoglobin in the red cell. Matthes' contribution was the continuous optical registration of light absorption in different spectral regions, using red light for oxygen content and green light for the total hemoglobin. Kramer's method¹⁰ for closed blood vessels in the experimental

* Received for publication, September 29, 1947.

animal was adapted in 1935 by Matthes¹³ to human beings, using an ear lobe attachment.

In 1939, workers in the Henry Ford Hospital laboratory imported and used the Kramer equipment. Experimental work led to the development of a method,⁵ the validity of which was tested through simultaneous recordings of blood oxygen saturation from transilluminated tissue and through the unopened femoral artery using Kramer's technic. This oxyhemoglobinograph* consisted of a photo-electric colorimeter, in which the beam of light passed through the first interdigital fold of the hand. The transmitted light fell on a high vacuum photocell, the D.C. output of which was amplified and was then indicated on a recording milliammeter. This apparatus provided the desired sensitivity and a considerable amount of clinical work was carried out with the unit. However, it exhibited troublesome drift characteristics due in part to the instability of the direct current amplifier. To circumvent this difficulty, the electron stream in the photocell was modulated³ in such a way as to produce an alternating current, which could be amplified with circuits of greater inherent stability. Then the most serious remaining limitation of this monochromatic method was the drift of the base reference due, it is believed, to four variables: (1) tissue thickness which affects the amount of light absorption by the tissue itself; (2) extent of the capillary bed which affects the amount of blood in the optical path; (3) total hemoglobin which affects the amount of hemoglobin in the optical path; and (4) vasomotor changes, caused by fluctuations in blood pressure which would affect factor (2), and associated at times with a redistribution of hemoglobin, as may occur in shock, which would affect factor (3). All of these variables will hereafter be included in the expression "volume variables".

Other workers^{4, 12, 14, 16} have also recognized the disturbing effects of these "volume variables" and have attempted to correct for them. Matthes eliminated local vasomotor changes by histamine iontophoresis to both ears. Simultaneous tracings from the photocell on one ear and a plethysmograph on the other allowed an immediate recognition of distortions in the oxygen saturation curve, caused by volume changes. In recent work, procedures have been planned to correct for these "volume variables" from one subject to another as well as in the same individual. The details of the correction method vary, but all involve a second spectral region, sensitive to total hemoglobin (near infra-red,¹⁶ blue,⁴ green^{12, 14}). Squire and Goldie standardized their instruments by rendering the tissue bloodless, whereas Millikan compared the fully flushed ear with an optical filter. To date, only Goldie,⁴ through the use of a ratiometer, and Hemingway and Taylor,⁷ through the use of "automatic compensation" suggested by Milli-

* This term was coined by Hartman and McClure. Since then, "oximeter"^{7, 14} and "anoxia photometer" (Coleman Electric Co.) have been used; the former denoting oxygen measurement and the latter denoting a photo-electric determination of lack of oxygen. Neither term bears any reference to blood or hemoglobin. Therefore, "oxyhemoglobino-graph" should be preferred, since it means a written record of oxyhemoglobin, and the tracing shows variations in the oxygenation of the hemoglobin over a wide range (60-100 per cent saturated). Therefore, this improved instrument will be called an "oxyhemograph", a logical contraction, since the older term "oxyhemoglobinograph" is unwieldy.

kan,¹⁴ claim that vasomotor changes are ruled out as a source of error. The method used in the present instrument will be described in detail later.

In the method presented herein an effort has been made to combine the best features of the technics described in these earlier publications. A number of rather general conclusions with regard to the best features of design will be stated before describing the method in detail.

The site of application should be a tissue area which is vascular but thin enough for light transmission and anatomically constructed so as to be placed in the path of the light with ease. The hand applicator has the disadvantage of limiting the sphere of activity as well as creating fatigue in the subject because of its encumbrance. Experimentation with our oxyhemoglobinograph showed that the state of muscular relaxation or tension in the hand and arm had a disturbing effect on the base reference. Since Hertzman⁸ has shown that the ear participates only weakly in vasomotor reactions, whereas the hand shows definite respiratory vasomotor changes, it is obvious that the ear attachment should have the advantage.

The photocell selected may be either the high-vacuum emissive phototube or the barrier-layer photocell, but the latter is much more compact and is therefore better adapted for use on the ear.

The amplifier must be adapted to the cell, be stable, and have sufficient output to operate a rugged recorder. One objection to the barrier-layer photocell has been the difficulty of maintaining a high degree of stability in a system, subject to conventional D.C. amplification.

A recording instrument such as the Esterline-Angus milliammeter has the advantage of furnishing a continuous ink tracing of the amplified current as well as being rugged, portable and quick in response.

METHOD

The complete apparatus consists of a Millikan "lamp-filter-photocell" ear unit, a battery (6 volt) for the light source, the contact-modulated direct current amplifier, an amplifier power supply (regulated), and an Esterline-Angus recording milliammeter. The ear unit contains two miniature photocells, a small lamp and two color filters. Light transmitted through the pinna of the ear is measured in two spectral regions. The "red" cell photocurrent reflects changes in the oxygenation of the blood together with the volume variations mentioned above. The "green" cell photocurrent reflects only "volume variables" because in this spectral region the absorption characteristics of oxyhemoglobin and reduced hemoglobin are similar. The two photocurrents are connected in opposition to produce a difference signal in which the changes due to volume variations are largely canceled out, leaving only the fluctuations due to a shift in the degree of oxygenation.* This difference signal is broken up by the input breaker to a pulsating current (80 cycles per second) which is amplified by a conventional

* Instead of taking the difference between these two individual photocurrents, their ratio might be measured as was done by Goldie.⁴ This may have some advantages, but has not been used in our work.

resistance-coupled alternating-current amplifier. The amplified signal is then rectified by an output breaker synchronized with the input breaker, to produce an unidirectional output current. This output is sufficiently large to actuate the recording milliammeter. A view of the amplifier incorporating the bichromatic photocell control circuits and lamp control circuits is shown in Figure 1 together with the recording milliammeter and Millikan earpiece. The various parts of this system will now be described in more detail.

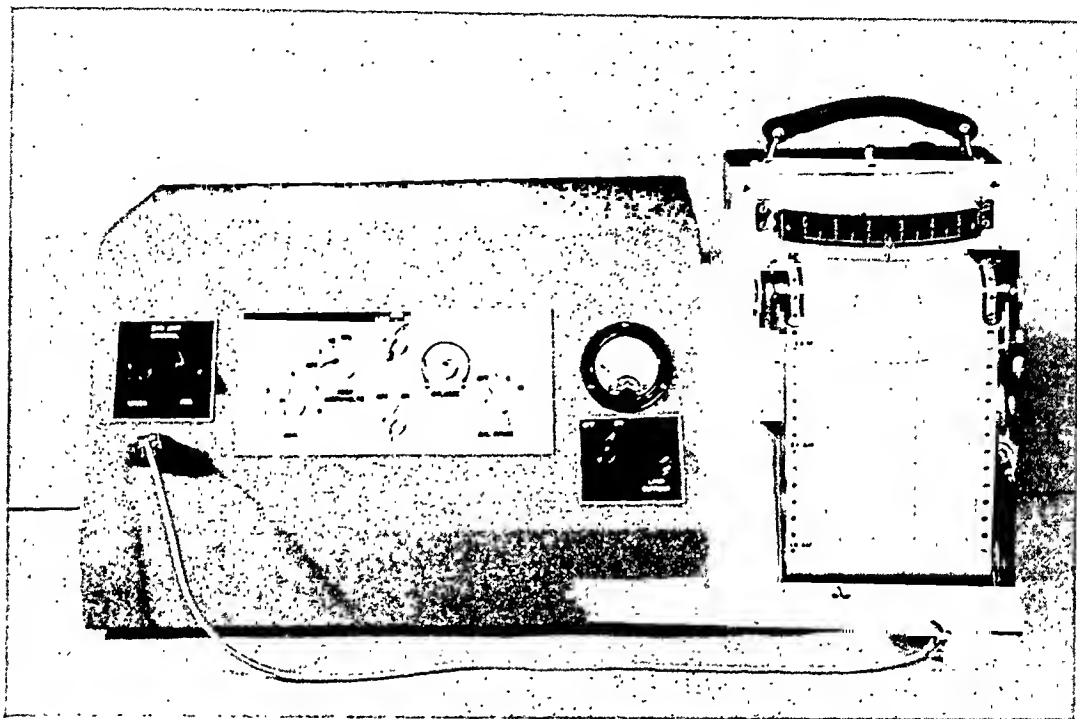


FIG. 1. PHOTOGRAPH OF AMPLIFIER UNIT, RECORDING MILLIAMMETER, AND EARPIECE

The Control Circuit

The control potentiometers are mounted on the amplifier panel as may be seen in Figure 1. The essential features of automatic compensation for "volume variables" are incorporated in the photocell control circuit shown in Figure 2. The earpiece* contains the miniature lamp placed on one side of the ear and the two photocells with their associated filters on the other. One portion of the transmitted light passes through the green (Wratten #61) filter striking the photosensitive surface of the "green" photocell to produce a voltage across the "green" potentiometer, the polarity of which is indicated in Figure 2. The other portion of the light transmitted by the ear traverses the red (Wratten #29) filter before striking the "red" photocell and produces a voltage across the "red" control potentiometer with polarity as marked in the diagram.

The "green" photocell surface area is almost three times that of the "red" and its control potentiometer contains twenty times the resistance of the "red" con-

* Coleman Electric Company, Maywood, Ill.

trol potentiometer. The need for these values is occasioned by the light absorption of the ear. Since most of the transmitted light from the ear is in the visible red there is little transmission in the green region. The greater area of the "green" cell, and the higher resistance associated with it, allows an output voltage sufficiently large to balance the "red" cell output.

An inspection of the photocell and potentiometer circuits (Fig. 2) reveals that the two voltages produced by the photocells are connected in polarity opposition so that only their differences are transmitted to the amplifier. Each voltage, however, may be adjusted individually and, in operation, the "green" cell control is turned to deliver full voltage to the input circuit, while the "red" cell control is adjusted to produce an equal voltage. Under these conditions there is no output voltage to the amplifier. This initial adjustment is made with the ear-piece in place on the ear. Then if vasomotor changes occur, there will be a similar variation in both voltages and the output will remain at the initial zero setting corresponding to normal oxygenation. A change in oxygenation, however, produces a variation in the "red" photocell output only and the difference voltage obtained, corresponds to the alteration in the oxygen saturation. It is true that perfect compensation cannot be obtained with this differential indication; however, tests have shown that its use provides a stability quite satisfactory in most applications.

The Amplifier

The amplification system is shown in block diagram in Figure 3. The direct-current output voltage from the photocell control circuit is impressed alternately between the two halves of the input transformer primary by means of the cam-driven input breaker. The polarity of these windings is such that a resultant flux is developed to produce an alternating voltage in the secondary. The breaker frequency is controlled so that 80 complete alternations per second are obtained. Thus a transformation from direct current to alternating current is effected so that an A.C. amplifier may be used and the instabilities of conventional direct current amplification avoided.

An immediate amplification of the input voltage is obtained in the input transformer since the ratio of the primary to secondary turns is 1 to 14. The remaining amplification is secured in the three-stage amplifier. The amplified signal is then impressed upon the primary of the output transformer.

The output breaker accomplishes the reconversion of the amplified signal back to an unidirectional current for the recording instrument. This breaker is driven by a cam on the same shaft which actuates the input breaker. The rectification produces unidirectional current pulses but the departures from the steady state value are too rapid for the recorder pen to follow and the effects of pure direct-current output are obtained.

This method of converting the direct-current input signal into alternating current before amplification has very marked advantages over conventional direct-coupled D.C. amplifiers. In these conventional D.C. amplifiers the most troublesome instability is caused by variations in the tube electron streams.

These variations may be large when compared to an input signal and still have little effect on the amplification factor of the tubes. This source of instability is practically eliminated in the "contact modulated" amplifier since the operation depends only on the dynamic amplification factors of the tubes. Another advantage is derived from the breaker system. Synchronous rectification in the output circuit allows only those voltages of the same frequency as the input breaker to produce a direct current component in the output circuit. Thus voltages induced from stray fields, from electrical apparatus, and random amplifier voltages other than breaker frequency will produce only alternating current components in the output circuit, and will consequently be discriminated against by the integrating action of the recorder. A further safeguard against stray fields and other sources of random voltage variations was provided by tuning

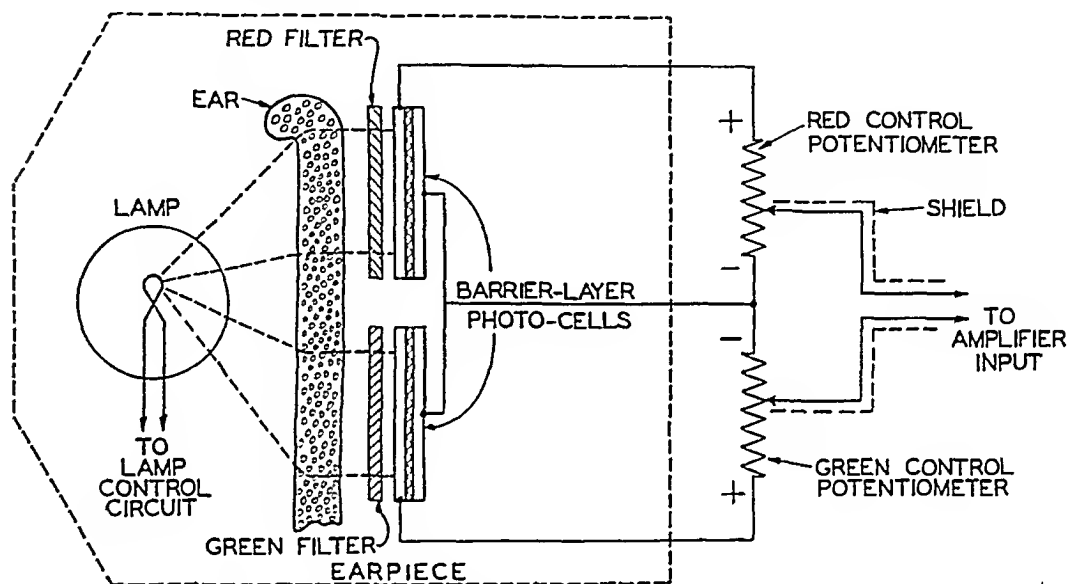


FIG. 2. CIRCUIT DIAGRAM OF THE PHOTO-ELECTRIC EARPiece AND CONTROL POTENTIOMETERS

the amplifier so that voltages with different frequencies from the breaker frequency are greatly attenuated.

The original amplifier design was dictated by the requirements associated with the needs for amplification of minute voltages from low-resistance thermocouples used in infra-red spectroscopy. Therefore, the input transformers were designed to operate from input sources of 5 to 20 ohms. The barrier-layer photocells together with the control potentiometers have a resistance of approximately 1500 ohms under normal operating conditions. This higher resistance requirement was met by increasing the number of primary turns on the input transformer.

A new difficulty was apparent when the amplifier was tested with the higher input impedance. The capacity variations associated with the motion of the input breaker arm which had caused no trouble when the original low impedance windings were used, now produced voltages of considerable amplitude. These troublesome induced voltages were practically eliminated by using an electro-

static shield between the transformer windings and by revising the input breaker circuit so that the breaker arm remained at ground potential as shown in Figure 3.

The amplifier may be operated at three sensitivity ranges. The sensitivity for each range is regulated by a continuously variable gain control. The test signal and balancing circuits of the original amplifier design were incorporated in the input circuit. These provide standard signals of 1, 10 and 100 microvolts which may be employed to check the amplifier gain. A continuously variable balancing voltage may be used to bias the zero to any desired position. These voltages are applied across a short length of one of the input transformer lead wires. All the amplifier controls appear on the central panel in Figure 1.

The lamp control units together with a milliammeter for monitoring the lamp current are located on the front panel at the right of the amplifier control group. The power supply for the amplifier is housed within a separate enclosure.

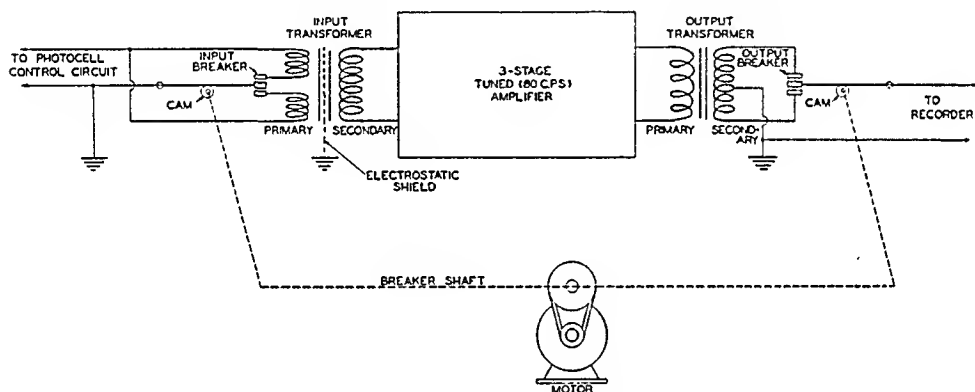


FIG. 3. BLOCK DIAGRAM OF THE AMPLIFIER UNIT

PROCEDURE

In the use of this amplifier a period of about fifteen minutes is required for an equilibrium to be reached. The recorder is mechanically adjusted to zero. In compensating for individual ear variations, the lamp current is turned on and set at 150 milliamperes with a test filter,* either A or 0.5 A, substituted for the ear in the photocell unit. With the "green" control full on and the "red" control off, the recorder pointer is adjusted to a full scale deflection by increasing the gain of the amplifier. The lamp current is then turned off. The test filter is removed and the ear slipped into the space between the lamp and the photocells. The earpiece should be placed over the anthelix of the pinna and should be fastened securely but not so tightly as to impair the circulation. The lamp is again turned on with a current of 150 milliamperes so as to transilluminate the ear. At least ten minutes should be allowed for the maximum warming effect on the blood vessels to be attained. The oxyhemograph pointer will assume a

* Coleman Electric Company, Maywood, Ill.

steady reading* when the greatest degree of vasodilatation is reached. The "red" cell is turned on and the resistance adjusted by the "red" control until the pointer is back to zero. The gain control is then turned to the operating position. A slight readjustment of the "red" control may be necessary to bring the pointer to zero. A test signal (10 microvolts) is introduced to record a deflection which enables the operator to verify and reduplicate the gain setting and thus employ

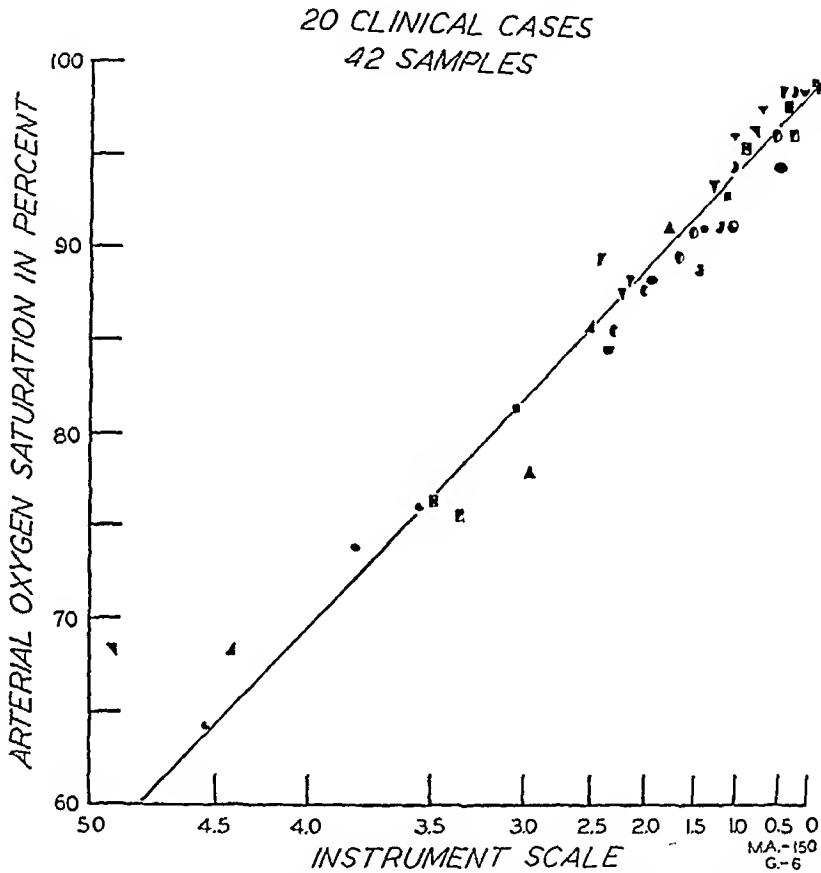


FIG. 4. Calibration data obtained under anesthesia. Analyses of arterial blood from one individual are represented by the same symbol. M. A. represents the lamp current in milliamperes. G. represents the operating position.

a known degree of amplification. The position of the pointer is indicative of the

* This reading is indicative of the comparative light transparencies of the ear and the filter. These data are being accumulated to study the possibility of classifying relative ear transparencies in a species as was done by Millikan.¹⁴ Such a classification might aid in the selection of the operating position, *i.e.*, the gain setting which would offer adequate sensitivity for changes in oxygenation. It would be ideal if the standardization procedure would allow compensation for the individual "volume variables" (1) to (3). In the human species, it was possible to use a fixed operating position, since there was fairly close agreement in the "ear transparency" values. In the dog, however, the variety of breeds used was responsible for a greater variation in "ear transparency" values. This necessitated the selection of different operating positions so that sufficient sensitivity would be maintained. Although this phase of the method remains under consideration, it is obvious that calibration data must be obtained on each species.

per cent oxygen saturation of the blood under the prevailing conditions (room air, approximately 95 per cent saturated). The pointer is set at the proper oxygen percentage on the scale by the mechanical zero adjuster, before the recorder is started. An increase in oxygenation of the blood will increase the light transmission, thus causing the pointer to approach zero (100 per cent saturation), whereas a decrease in oxygenation, through its diminished light transmission, will move the pointer toward 5 (60 per cent saturation) on the scale.

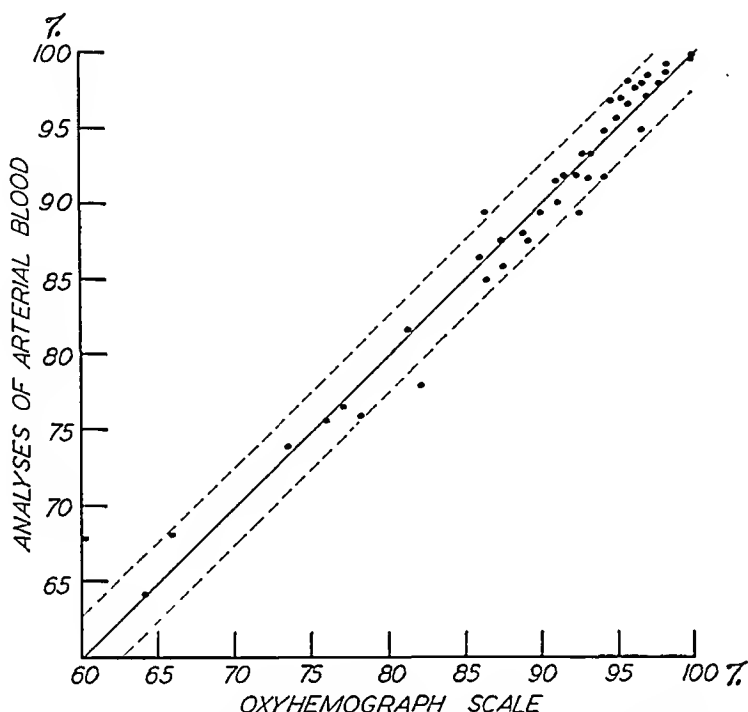


FIG. 5. Correlation between the chemical and instrumental values for the blood O_2 saturation in per cent. Identical values fall on the solid line. Twenty-eight out of 42 points (66.6 per cent) lie within ± 1 per cent. Those points (39 out of 42 or 92.8 per cent) included within the broken lines represent an agreement of $\pm 2\frac{1}{2}$ per cent. The remaining points (3 out of 42 or 7.2 per cent) are within 3-8 per cent.

CALIBRATION

The instrument was calibrated by correlating actual arterial blood oxygen saturation values with the oxyhemograph readings. Arterial blood was drawn under oil from the cannulated femoral artery in anesthetized dogs or from a puncture of the femoral artery in anesthetized human beings. The blood (5 cc.) was immediately injected into an air-evacuated oxalated bottle and refrigerated. The blood was analyzed in duplicate for oxygen content and oxygen capacity using Van Slyke's manometric method. The per cent saturation was computed from these figures.

From a study of Pentothal-nitrous oxide-oxygen anesthesia in 20 patients

undergoing orthopedic operations, blood oxygen values were obtained for a calibration curve on human beings.² Figure 4 represents the data obtained from 42 arterial punctures in 20 patients. In accordance with Beer's Law, the oxyhemoglobin concentration is related to the logarithm of the transmitted light. Therefore, the arterial oxygen saturation in per cent is plotted on the ordinate, while the instrument reading is plotted logarithmically on the abscissa. The

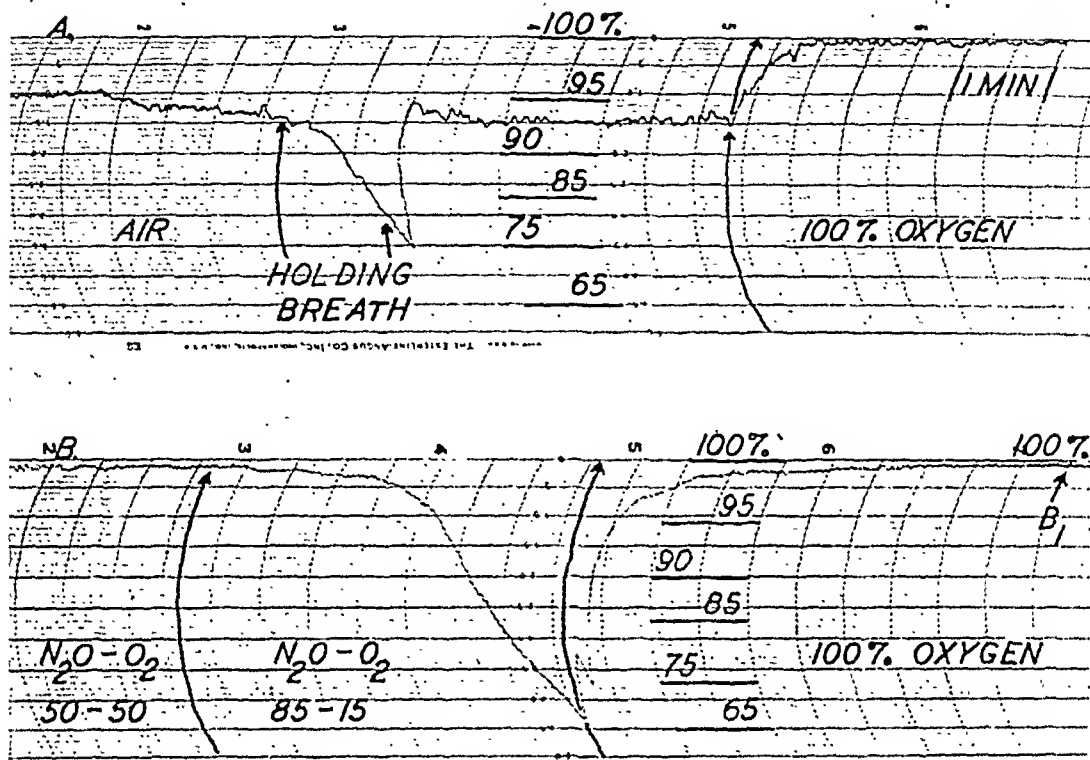


FIG. 6A. A continuous record of the blood oxygen saturation in per cent in a normal subject. The effect of holding the breath for 65 seconds in air, the recovery period, and of 100 per cent oxygen inhalation on the blood oxygen level is depicted.

FIG. 6B. An eleven-minute excerpt from the blood oxygen tracing during the first one-half hour of Pentothal-nitrous oxide-oxygen anesthesia on a clinical subject, undergoing an orthopedic operation. Patient received premedication of morphine, gr. 1/6 and atropine, gr. 1/150, followed by 0.85 gm. Pentothal. The tracing shows the efficiency of 50-50 nitrous oxide-oxygen and 100 per cent oxygen in maintaining an oxygen saturation of from 98 to 100 per cent. A four-minute administration of an 85-15 nitrous oxide-oxygen mixture, caused a steady decrease in the blood oxygen saturation to 68 per cent. B₁ represents an arterial puncture and its analysis.

straight line is plotted through an average of points on each ordinate and is used in assigning the per cent oxygen saturation values to the oxyhemograph scale.

The agreement between the blood oxygen saturations in per cent (calculated from analyses of 42 arterial blood specimens) and the calibrations assigned to the instrument scale is shown in Figure 5. Considering the errors associated with the chemical analyses and the changing oxygen content of the blood during the process of sampling, the correlation of 92.8 per cent of these two kinds of data within $\pm 2\frac{1}{2}$ per cent is significant. The conformity of the blood values and the oxyhemograph should be proof that the instrument is reliable.

The calibration of the oxyhemograph for use in the experimental animal (dog) has been complicated by variations of breeds within the species. In order to maintain adequate sensitivity, it was necessary to select operating positions ranging from gain 4 to gain 7. This called for a calibration at each operating position, since the degree of amplification is determined by the gain setting. Data at gain 5 showed a trend similar to that obtained clinically, although there

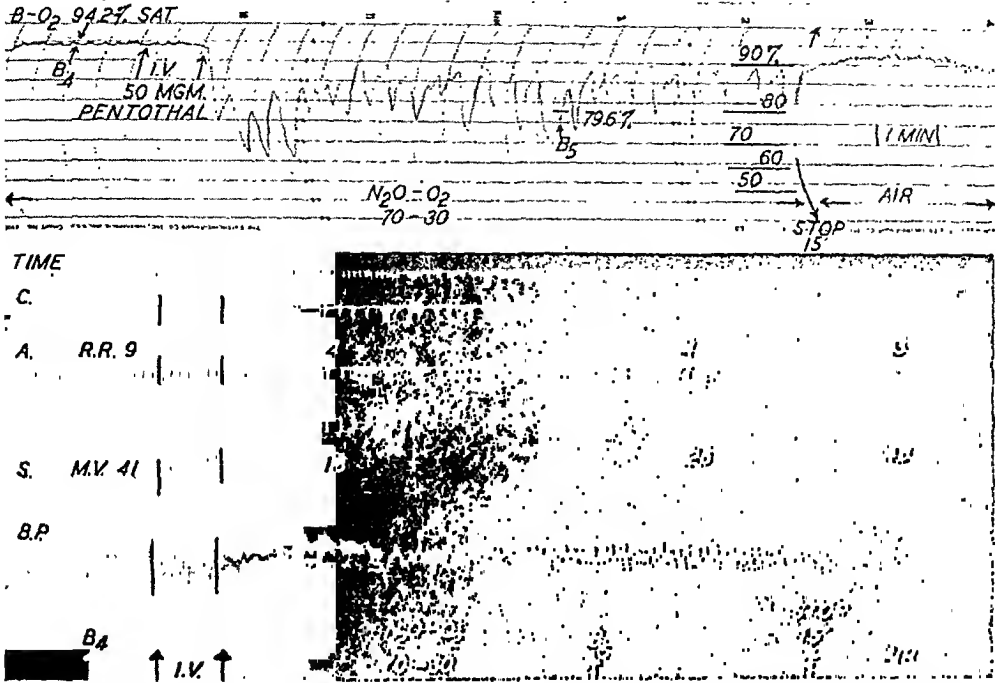


FIG. 7. Simultaneous tracings of the blood oxygen saturation in per cent (B-O₂), time in fifths of a minute, costal (C.) and abdominal (A.) respiration, spirometer (S.) record of pulmonary ventilation, and blood pressure (B.P.) on a dog, subjected to Pentothal-nitrous oxide-oxygen anesthesia are shown. B₁ and B₂ represent analyses of arterial blood. Respiratory rate (R.R.) and pulmonary ventilation per minute in deciliters (M.V.) are stated at intervals. An intravenous (I.V.) injection of Pentothal during the administration of a 70-30 nitrous oxide-oxygen mixture causes a depression in the respiration which is reflected in the blood oxygen level as the curve drops sharply toward 64 per cent saturation. As the expiratory phase of each respiratory cycle is completed, one observes an immediate upward swing of the pointer to a peak, followed by a fall until the end of the next respiratory cycle. It is possible to align each wave in the tracing with a breath, although two respirations, in close succession, lead to a double-peaked wave. After a fifteen minute period of breathing air, the more rapid respiration of lighter anesthesia results in a return of the blood oxygen saturation to normal with the respiratory effects on the tracing smoothing out.

was a wider distribution of points. The conformity of the analyses of dog blood and the oxyhemograph values, plotted as in Figure 5 on clinical data, was less precise. An analysis of these data revealed that 32 out of 45 values or 71.1 per cent were within the limits of $\pm 2\frac{1}{2}$ per cent. The remainder were dispersed over a wide range (3-20 per cent). This distribution may occur because the correlation between the "ear transparency" values and the operating position is not ideal. Although calibration data (88 bloods in 28 experiments) have been

accumulated during studies on intravenous anesthesia, ^{1,2} further evaluation of this aspect of the calibration is required.

APPLICATION

The development of a valid apparatus, such as this oxyhemograph, makes possible the study of varied problems in physiology and medicine which deal with the degree of oxygenation of the blood. It has already proved its worth in anesthesia studies, both clinical,^{2,5} and experimental,^{1,2} and in aviation research.⁶

The pharmacologist and anesthesiologist should find in the oxyhemograph an effective tool for the objective evaluation and selection of anesthetic agents. Observations on the normal and on the anesthetized subject have afforded data* on the effectiveness of oxygen inhalation as well as the effect of Pentothal, Pentothal-oxygen and Pentothal-nitrous oxide-oxygen anesthesia on the blood oxygen level. A record on a normal unanesthetized subject (Fig. 6A) and an excerpt from an operation (Fig. 6B) are illustrative of this type of data. Experimental studies, which include graphic recordings of physiologic data as well as the blood oxygen tracing, demonstrate the effects of respiratory changes on the blood oxygen level (Fig. 7).

A desirable feature of the oxyhemograph is the stable but rapid response to small differences in the blood oxygen. In fact, the sensitivity of the instrument permits a study of the fluctuations in the oxygenation of the blood associated with the respiratory cycle, as shown in Figure 7. This characteristic should make the instrument an invaluable aid to the anesthetist. The first warning of developing anoxemia is registered by this delicate monitor, much earlier than can be appreciated by clinical observations because the slightest change in the transparency of hemoglobin activates a photocell signal, which is amplified a million times before recording. Such high sensitivity, coupled with stability and portability is a combination of features which has not been attained previously.

The oxyhemograph should prove useful in the study of all conditions which may be related to anoxemia and anoxia, such as edema or consolidation of the lungs, hydrothorax or pneumothorax, reduced absorption of oxygen due to decreased atmospheric pressure, congenital anomalies of the heart and blood vessels, paralysis of respiratory muscles, cardiac insufficiency and shock.

SUMMARY

An accurate, stable and sensitive continuous photo-electric method for recording the per cent oxygenation of the blood is described. The apparatus, as well as the mode of operation, is described in detail. Calibration data on clinical cases show that the chemical and instrumental data are usually within $\pm 2\frac{1}{2}$ per cent. The application and sensitivity of this instrument is discussed.

Acknowledgments. The continued personal interest of Mr. C. F. Kettering, who made available the facilities of the Research Laboratories Division of the General Motors Corporation for the engineering phases of the work, and of Dr. R. D. McClure, who encouraged observations on surgical patients at the Henry Ford Hospital, has made this study possible.

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HEMANGIOMA OF THE SMALL INTESTINE

WITH SPECIAL REFERENCE TO INTUSSUSCEPTION. REVIEW OF THE
LITERATURE AND REPORT OF THREE NEW CASES*

PAUL SCOTT HANSEN, M.D.†

Hemangioma of the small intestine is a rare condition but is important because it can cause intussusception, hemorrhage and obstruction and an array of puzzling gastro-intestinal symptoms challenging the acumen of the diagnostician. An understanding of its clinical features should lead to its more frequent recognition preoperatively or ante mortem.

INCIDENCE

Tumors of the small intestine are rare and, of these, hemangiomas are among the rarest. In 11,500 autopsies and 45,000 surgical specimens, Raiford⁵¹ found 986 tumors of the entire gastro-intestinal tract, of which 88 were in the small intestine. Fifty of the 88 tumors were benign and 3 were hemangiomas. Merchant⁴¹ found 24 benign tumors of the small intestine in 7340 autopsies and 50,775 surgical specimens. Eighteen of these were found at autopsy and 6 at operation; of the 24 tumors, 3 were hemangiomas. During the same period a total of 274 benign tumors of the intestinal tract from cardia to anus was found. Willis⁶¹ found 19 benign tumors of the small intestine in 7492 autopsies at the Massachusetts General Hospital and the Boston City Hospital. He did not state whether there were any hemangiomas. King³² could find only one case of small intestinal tumor in the reports of 44,654 intraperitoneal operations at the Mayo Clinic up to 1917. In 1933, Rankin and Newell⁵³ reported 95 cases of small intestinal tumor from the same clinic. Thirty-five of these were benign and 2 were hemangiomas. Raiford's⁵¹ figures are the most complete. In his series, tumors of the small intestine comprised 8.9 per cent of all gastro-intestinal tumors, and benign tumors of the small intestine comprised 5.07 per cent of all gastro-intestinal tumors. The incidence of hemangiomas was 6 per cent of benign tumors of the small intestine, 3.4 per cent of all tumors of the small intestine and 0.3 per cent of all gastro-intestinal tumors. In Merchant's⁴¹ series, hemangiomas comprised 12.5 per cent of benign tumors of the small intestine.

Of 51,261 admissions to the surgical services of the Hospital of the University of Pennsylvania from 1922 to 1940, there were 28 cases of small intestinal tumor. In 24 cases the tumor was malignant; in 4 cases it was benign. There were no cases of hemangioma.

FREQUENCY OF INTUSSUSCEPTION

Intussusception is the commonest cause of serious acute abdominal disease in infants under 2 years of age. According to Fiske,²⁰ primary intussusception in

* Received for publication, August 2, 1947.

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adults and older children is very rare, although he cites Goodall²² as having collected 122 cases. The causes of secondary intussusception are tumors, benign and malignant, ulceration due to tuberculosis, dysentery and typhoid, and Meckel's diverticulum. Benign tumors produce intussusception much more frequently than malignant tumors. Rankin and Mayo⁵² found intussusception in only 2 of 55 cases (3.6 per cent) of carcinoma of the small intestine. Rankin and Newell⁵³ found intussusception in 17 per cent of their series of 35 benign tumors (and in 33 per cent of those cases which produced symptoms), and Merchant⁴¹ in 7 (29 per cent) of his series of 24 cases of benign small intestinal tumor.

Joyce²⁶ stated that intussusception is a complication of more than 30 per cent of small intestinal tumors, and is much commoner with benign tumors. It occurred in 2 of his 4 cases of benign tumor and in none of his 5 cases of malignant tumor. Intussusception was a feature of all 3 of Willis⁶¹ cases of benign tumor of the small intestine. Thus, benign small intestinal tumors are the commonest cause of clinically apparent intussusception of the small intestine in the adult.

While the above figures show that from 15 to 30 per cent or more of benign small intestinal tumors cause intussusception, it is noteworthy that hemangiomas, in general, relatively seldom cause this condition. The reasons for this become apparent when the classification of hemangiomas according to their gross morphologic appearance is considered.

ORIGIN AND TYPES OF HEMANGIOMA

The origin and nature of hemangiomas have been ably presented by Kaijser²⁹ in his thorough article which I shall briefly summarize. The major theories are: (1) That of Ribbert, which is based on Cohnheim's theory of embryonic rests and which postulates that hemangiomas are congenital rests which may or may not later show autonomous growth. Von Hippel's and Lindau's disease may be the result of later autonomous growth in such a structure; (2) That hemangiomas are the result of a congenital mesodermal defect (status varicosus) involving the veins and capillaries with a tendency to dilatation of the weak vascular walls. Rendu-Osler's disease (hereditary familial telangiectasis) is suggested as being of this type; (3) That irritation, as in the gastro-intestinal canal, may produce dilatation of already formed vessels and some new formation; (4) That the racemose type may be due to an abnormal arteriovenous communication. It is questionable whether this type can be considered neoplastic and, in any event, no instance of this type has been described in the gastro-intestinal tract.

Kaijser^{29,30} believes that most hemangiomas are congenital abnormalities which lack the capacity for autonomous growth and therefore should not be considered true neoplasms. His reasons are as follows: (1) they are generally present at birth; (2) they are often familial; (3) they are often widespread in a given individual; (4) they are occasionally associated with other malformations.

There is no doubt, however, that some hemangiomas do show autonomous growth and are true neoplasms. Malignancy seems to be very rare, although it does occur. Ewing¹⁹ refers to a "congenital angiosarcoma of the ileum" reported by Stern in 1894 and Kaijser²⁹ mentions another case of hemangiosarcoma of the

small intestine reported by Ackerlund. Sarcomatous changes have also been noted in hemangiomas of the skin. Such cases with metastases are difficult to differentiate from cases with multiple primary lesions.

Morphologically, hemangiomas may be classified grossly and microscopically. Clinically, the former classification is the more important. Microscopically, hemangiomas fall into three types, as follows:

1. Simple, consisting of new formed capillaries which may or may not be dilated with little or abundant fibrous stroma;
2. Cavernous, consisting of large blood-filled spaces, lined with a single layer of endothelial cells and communicating with one another with little or no stroma;
3. Combined, consisting of a combination of the above types. Any of these types may be either solitary or multiple. The degree of endothelial hyperplasia determines whether the growth should be considered an hemangioma, hemangio-endothelioma, or endothelioma.

Brown's¹¹ gross classification of gastro-intestinal hemangiomas is simple and practical and has been widely quoted in the American literature on the subject. However, since Kaijser's adaptation of Oberndorff's classification is more detailed and more applicable clinically it is used in this paper, as follows:

- I. Multiple phlebectasia
- II. Cavernous hemangioma
 - A. Diffuse infiltrating cavernous hemangioma
 - B. Circumscribed, often polypoid, cavernous hemangioma
- III. Simple capillary hemangioma (angioma simplex)
- IV. Angiomatosis localized in the gastro-intestinal tract.

Each type is considered separately on the basis of the 66 cases reported in the literature since 1860 including the 3 reported here for the first time.

Type I. Multiple Phlebectasia

As the name implies, this consists of multiple dilated venous structures, microscopically usually of the cavernous type, appearing sometimes in large numbers as pinhead to pea-sized purple nodules. Generally they may be seen connected with a vein. They seldom cause symptoms and are usually discovered by accident at autopsy. These lesions may represent the same pathologic entity as Rendu-Osler's disease, but hemorrhage is very rare and the congenital factor has not been demonstrated in the gastro-intestinal cases. Twenty-seven of the reported 66 cases were of this type; 24 were in males and only 3 in females. The age incidence was from 34 to 79 years, one-third of the cases occurring in patients in their seventh decade. The lesions were widespread throughout the small intestine in most of the 27 cases. In at least 6 instances, other parts of the gastro-intestinal tract were specifically mentioned as being involved. The jejunum appears to be the commonest site in the small intestine as it was mentioned by name in 15 cases and was implied in most of the others.

The lesions were said to be in the submucosa alone in 18 of the cases; in 2 cases the submucosa and muscularis were both involved; 2 cases appeared to show only

subserosal lesions and in 4 cases the portion of intestinal wall involved was not stated.

In 18 cases there were no symptoms, while in 4 cases symptoms were not mentioned. In 4 cases there was hemorrhage which was accompanied in only 1 instance by abdominal pain. In 1 case there was hemorrhage which was attributed to gastritis.

In the 4 cases with hemorrhage from the intestines exploratory laparotomy was carried out. In no case was operation of more than diagnostic value. In 7 cases the cause of death was not stated; of the other cases, in only 1 instance was death caused by hemorrhage.

Type II. Cavernous Hemangioma

A. Diffuse infiltrating cavernous hemangioma. This type of hemangioma infiltrates diffusely and replaces a limited portion of the intestinal wall. The wall is thereby thickened and the lumen is usually narrowed. The cavernous tissue usually invades all layers, occasionally sparing the mucosa and muscularis mucosae. This lesion also often affects the rectum but only rarely does it affect the stomach. Both sexes are about equally affected. Symptoms often appear in early youth and consist of hemorrhage and obstruction. Phleboliths are said to be common and are often an important diagnostic point, but in no reported case of this type has the presence of phleboliths been mentioned.

This report includes 12 instances of this type; 7 were in females, 5 in males. The age incidence ranged from 3 months to 58 years. Six of the patients were 21 years of age or younger and only 2 were over 40. In all but one of the cases, that of Pierose,⁵⁰ the lesions were probably solitary, although in some instances so extensive as to suggest multiplicity. The jejunum and ileum were about equally affected and the duodenum was affected less often.

All of the patients in this group had symptoms; hemorrhage occurred in all but 4 patients. A mass was felt in 1 patient, a 3 month old child. Symptoms of obstruction were encountered in 6 patients, or in 50 per cent. Resection was carried out in 9 persons and exploratory laparotomy in 1. In 6 instances the resection resulted in cure, while 1 person died of postoperative complications. The other 2 patients died of obstruction in spite of operation. Death in the 2 unoperated patients was due, at least in part, to the hemangioma.

In most of these cases the tumor was annular, with constriction of the lumen at autopsy or operation in 8 cases.

B. Circumscribed, often polypoid, cavernous hemangioma. Eleven of the 66 tumors were of this type. They are always single and always cavernous. They vary in size from a few millimeters in diameter to several centimeters. It may be said that they give the impression of being true tumors capable of autonomous growth, whereas the diffuse infiltrating cavernous hemangiomas give the impression of being congenital malformations. These circumscribed cavernous hemangiomas not infrequently cause hemorrhage and, when polypoid, lead to the same

symptoms as those produced by other polypoid tumors, notably obstruction and intussusception. In 3 of these 11 cases, intussusception occurred.

The lesion is usually submucosal but may be subserosal or involve all layers. The incidence in the sexes is equal and the age incidence is from 15 to 72 years. The tumor was the direct or indirect cause of death in 3 instances. Resection was carried out in 6 patients and recovery followed in all but one, in which death occurred from postoperative pneumonia.

Type III. Simple Capillary Hemangioma

Eight of the 66 cases of hemangioma were of this type. In 5 cases the lesion was single, in 2 multiple and in 1 the number of lesions was not stated. This type differs from those previously mentioned only in that the capillaries are not grossly dilated, and, like the foregoing type, gives the impression of being a true neoplasm capable of autonomous growth, results in obstruction and tends to cause intussusception. The most notable histologic feature is the presence of large numbers of fine, densely packed capillaries with relatively sparse stroma, consisting of cells which appear to be derived from the capillary endothelium. This type represents a transition to hemangio-endothelioma. This tumor is usually spherical and projects into the lumen of the intestine. Typically, it is the size of a plum.

Five of the 8 cases occurred in patients under 23 years of age. One occurred in a patient of 60. The sexes were equally represented. The size of the tumors in instances where size was recorded varied from that of a "lentil" to $9.5 \times 3 \times 3$ cm. In 5 of the 8 cases, the tumor was found in the ileum. In at least 5 cases, the tumor was in the submucosa and in 1 of these it appeared to invade other layers as well. All 5 of these cases were among the 6 in which clinical symptoms were reported. Hemorrhage was reported in 2 cases together with symptoms of obstruction. In 4 patients symptoms of obstruction occurred alone. Intussusception occurred in 2 of the 6 patients reported to have symptoms. In another 2, the symptoms of indigestion, pain and vomiting were not diagnostic of obstruction except, perhaps, in retrospect.

Operation was performed on 7 patients. All but one recovered; the death resulted from obstruction in spite of ileostomy. In all the successful cases, extirpation or intestinal resection was performed.

Type IV. Hemangiomatosis Localized in the Gastro-Intestinal Tract

In this condition small intestinal hemangiomatosis is simply an incident (although it may be a most important incident clinically), in more or less widespread hemangiomatosis involving particularly the skin and the liver. Any or all of the types of hemangioma previously described may be found, with diffuse infiltrating cavernous hemangioma or multiple phlebectasia predominating. In some instances the lesions are present at birth; in others they may appear or

enlarge later. It is thought that their development may be associated in some patients with pregnancy.

Microscopically, besides the types described above, there may be some in which proliferation of the endothelial elements is so great as to merge into and suggest angiosarcoma. It is difficult or impossible to say whether some lesions may not represent metastases.

Hemangiomatosis of the gastro-intestinal tract is rare compared with hemangiomatosis elsewhere but doubtless many cases are not discovered, since the condition is usually asymptomatic. When a symptom does occur, it is usually a violent and uncontrollable hemorrhage and there is a very high mortality rate. Operation is generally of no avail. Only 8 clear-cut cases of hemangiomatosis involving the small intestine have been reported. In all but the one reported here for the first time, symptoms were produced either by the hemangioma in the small intestine, or by similar lesions elsewhere. The patients varied in age from 4 to 81 years. Three were male and 5 were female. There seemed to be no special site of predilection in any part of the small intestine or of its wall. In at least 6 instances the submucosa was among the layers involved, doubtless explaining the high incidence of hemorrhage.

In 3 patients there were no gastro-intestinal symptoms. In the other 5, obstruction alone appeared once, and hemorrhage with or without other gastro-intestinal symptoms appeared in the rest. In 2 of these 4 patients hemorrhage was the cause of death in spite of operation. In 1, excision of a circumscribed cavernous hemangioma from the duodenum resulted in improvement. Intussusception was recorded twice in this group. In one case it was probably agonal. In both instances there was a polypoid hemangioma at the site of the intussusception.

COMMENT

The diagnosis of any type of hemangioma of the small intestine is rarely made preoperatively partly because of its rarity and partly because the condition seldom produces symptoms. The diagnosis will be made more frequently if the condition is kept in mind and if due attention is paid to the following: (1) the presence of hemangiomas on skin or mucous membrane surfaces; (2) the frequent presence, demonstrable by x-ray, of phleboliths, homogeneous, or layered, in diffuse infiltrating cavernous hemangiomas; (3) the significant association of intussusception with polypoid hemangiomas of the small intestine; (4) the possibility of demonstrating indentations and constrictions of the small intestinal lumen by x-ray in cases of otherwise unexplained gastro-intestinal hemorrhage.

TAB
REPORTED CASES OF HEMANG

NUMBER	AUTHOR	DATE	AGE	SEX	TYPE I, MULTIPLE PHLEBECTASIA			
					Single or Multiple	Portion of Small Intestine	Portion of Wall Involved	Symptoms
1.	de Boyer ¹⁰	1877	62	M	Multiple	Lower jejunum and upper ileum	Submucosa	None
2.	Hektoen ²⁴	1900	48	M	Multiple	Upper third	Submucosa	None
3.	Mac-Callum ³⁹	1906	54	M	Multiple		Submucosa with some invasion of muscularis	Probably none; hemorrhage 2 years previously, possibly from gastritis
4.	Bennecke ⁶	1906	52	M	Multiple		Submucosa	None
5.	Ohkubo ⁴⁷	1907	66	F	Multiple	Jejunum	Submucosa	None
6.	Ohkubo ⁴⁷	1907	79	M	Multiple		Submucosa	None
7.	Raiford ⁵¹	1932	54	M	Multiple	Jejunum	Not Stated	None
8.	Merchant ⁴¹	1939	64	M	Multiple	Jejunum	Submucosa	None
9.	Brulé, Hillemand and Hamburger ¹³	1934	65	M	Multiple	Duodenum	Not stated	Abdominal pain; hemorrhage
10.	Ackerman ¹	1937	64	M	Multiple	Ileum and jejunum	Submucosa	None

TYPE I, MULTIPLE PHLEBECTASIA

Treatment	Outcome	Cause of Death	Autopsy	Character of Tumor	
				Gross	Microscopic
	Death	Pneu- monia	Yes	Jejunum and first part of ileum had nearly 50 tumors per sq. cm.; tumors rounded and up to size of a pea; appeared to be continuous with arterioles	Capillary vessels twisted together
	Death	Endocar- ditis of aortic and mitral valves	Yes	Saccular and globular dilatations of numerous enlarged veins	Dilated venules
	Death	Arterio- sclerosis, broncho- pneumo- nia, acute alcoholism	Yes	Multiple nodules 7 to 8 mm. in diameter in course of veins	Cavernous heman- gioma
	Death	Tubercu- lous men- ingitis	Yes	Lesions in esophagus, stomach and both small and large intestines	Cavernous phle- bectasia
	Death	Pneu- monia	Yes	Innumerable varices in submucosa of jejunum	Cavernous angioma
	Death	Pneu- monia	Yes	Same as preceding case but involving entire small intestine; largest nodule 0.5 cm. in diameter	
	Death	Broncho- pneu- monia	Yes	Multiple hemorrhagic nodules along course of veins	Cavernous heman- gioma with large sinuses filled with blood
	Death	Hyper- tensive heart dis- ease	Yes	Innumerable small submucosal cavernous hemangiomas in jejunum	
Exploratory laparotomy	Un- changed		No	Small scattered angiomatous nodules of small intestine as far as jejunum; also of colon	
	Death	Carcinoma of larynx with sepsis	Yes	Six endothelial lined spaces filled with blood in submucosa; lesions varied from few mm. to 1 cm. in diameter	Cavernous heman- gioma

TABLE 1—

NUMBER	AUTHOR	DATE	AGE	SEX	TYPE I, MULTIPLE PHLEBECTASIA			
					Single or Multiple	Portion of Small Intestine	Portion of Wall • Involved	Symptoms
11.	Bensaude, Hillemand and Génestoux ⁷	1935	55	F	Multiple		Not stated	Hemorrhage and diarrhea
12.	Dudley ¹⁸	1934	56	M	Multiple	Jejunum and ileum	Apparently in sub-serosa	Hemorrhage
13.	Decastello ¹⁶	1939	34	F	Multiple	Upper jejunum	Submucosa	Hemorrhage
14.	Amundsen ²	1938	65	M	Multiple		Submucosa and somewhat in muscularis	None
15.	Hansen	1947	59	M	Multiple		Probably submucosal	None
16.	Thierfelder* ⁶⁰	1873	48	M	Multiple	Jejunum and ileum	Submucosa	None
17.	Thierfelder* ⁶⁰	1873	45	M	Multiple		Submucosa	None
18.	Lilie* ³⁸	1879	65	M	Multiple	Jejunum and ileum	Submucosa	None
19.	Lilie* ³⁸	1879	55	M	Multiple	Jejunum	Submucosa	None
20.	Lilie* ³⁸	1879	66	M	Multiple	Jejunum	Subserosa	None

* Quoted from Kaijser; ²⁹ original reference not consulted.

TYPE I, MULTIPLE PHLEBECTASIA

Treatment	Outcome	Cause of Death	Autopsy	Character of Tumor	
				Gross	Microscopic
Exploratory laparotomy	Death	Not stated	Yes	Multiple hemangiomas less than 3 mm. in diameter; also in large intestine; no signs of hemorrhage or ulceration at autopsy	Cavernous hemangiomas
Exploratory laparotomy	Unchanged		No	Multiple dark blue and purple masses 1 to 5 mm. in size, singly and in groups, continuous with blood vessels	
Exploratory laparotomy	Unchanged after operation; died later	Hemorrhage	Yes	Multiple hemorrhagic and spongy hemangiomas the size of lentils; also mesenteric lymphangiectasis	
	Death	Pneumonia	Yes	Multiple nodules up to size of a pea, protruding somewhat into lumen of esophagus and entire small and large intestines	Large cavernous spaces
	Death	Hemolytic streptococcus septicemia and uremia	Yes	Small, firm rounded nodules consisting of blood clots in wall throughout small intestine; also two thrombosed veins in sigmoid	Cavernous hemangioma
	Death	Trauma	Yes	Telangiectases	
	Death	Variola	Yes	Telangiectases	
	Death	Pulmonary tuberculosis	Yes	Phlebectasia	
	Death	Carcinoma of colon	Yes	Lesions also in colon	Phlebectasia
	Death	Bronchopneumonia, senile gangrene	Yes	Lesions also in pylorus	Phlebectasia

TABLE 1—

NUMBER	AUTHOR	DATE	AGE	SEX	TYPE I, MULTIPLE PHLEBECTASIA			
					Single or Multiple	Portion of Small Intestine	Portion of Wall Involved	Symptoms
21.	Orff* ⁴⁶	1880	63	M	Multiple		Submucosa	None
22.	Möller* ⁴³	1916	47	M	Multiple		Submucosa	Not stated
23.	Möller* ⁴³	1916	46	M	Multiple	Jejunum and ileum	Submucosa	None
24.	Möller* ⁴³	1916	35	M	Multiple	Entire small intestine, mostly in jejunum	Submucosa	None
25.	Schmincke* ⁵⁵	1924	42	M	Multiple		Submucosa	Not stated
26.	Staemmler* ⁵⁸	1924	65	M	Multiple	Jejunum and ileum	Submucosa	Not stated
27.	Staemmler* ⁵⁸	1924	67	M	Multiple	Entire small intestine	Not stated	Not stated

* Quoted from Kaijser;²⁹ original reference not consulted.

NUMBER	AUTHOR	DATE	AGE	SEX	TYPE II A, DIFFUSE INFILTRATING CAVERNOUS HEMANGIOMA			
					Single or Multiple	Portion of Small Intestine	Portion of Wall Involved	Symptoms
28.	Delbet† ¹⁷	1899	21	F	Single		Submucosa and muscularis	Chronic intestinal obstruction
29.	Roedelius ⁵⁴	1923	36	F	Probably single	Lower jejunum	All layers except mucosa	Intermittent obstruction, no hemorrhage
30.	Brown ¹¹	1923	12	F	Single	Probably high in jejunum	Submucosa and muscularis	Acute intestinal obstruction
31.	Pierose ⁵⁰	1940	40	F	Multiple	Jejunum and ileum	All layers	Hemorrhage, first occurred when patient was 3 days old

† Case quoted from Brown;¹¹ original reference not available.

TYPE I, MULTIPLE PHLEBECTASIA

Treatment	Outcome	Cause of Death	Autopsy	Character of Tumor	
				Gross	Microscopic
	Death	Sepsis	Yes	Lesions also in colon	Varicosities
	?		?	Most lesions in small intestine	Phlebectasia
	Death	Trauma	Yes	Phlebectasia	
	Death	Cancer of esophagus	Yes	Phlebectasia	
	Death	Not stated	Yes	Phlebectasia	
	Death	Not stated	Yes	Phlebectasia	
	Death	Not stated	Yes	Phlebectasia	

TYPE II A, DIFFUSE INFILTRATING CAVERNOUS HEMANGIOMA

Treatment	Outcome	Cause of Death	Autopsy	Character of Tumor	
				Gross	Microscopic
Resection	Death	Obstruction	Not stated	Annular angioma with stricture	
Resection	Recovery			Occupied 15 cm. of lower jejunum; lumen was "hardly the size of a lead pencil"	Multiple cavernous hemangiomas
Resection	Death	Obstruction	Yes	Annular lesion 5 inches long forming constriction which would not admit tip of little finger	Cavernous hemangioma
Resection of 3 feet of jejunum	Recovery			Diffuse telangiectatic cavernous hemangioma of jejunum and telangiectases of duodenum	Large endothelium-lined spaces containing blood found in subserosa and submucosa of jejunum

TABLE 1—

NUMBER	AUTHOR	DATE	AGE	SEX	TYPE II A. MULTIPLE PHLEBECTASIA			
					Single or Multiple	Portion of Small Intestine	Portion of Wall Involved	Symptoms
32.	Peyton ⁴⁹	1938	15	M	Single?	All of jejunum and possibly some of the ileum	Not stated	Hemorrhage
33.	Carbonnel Salazar ^{*14}	1939	12	F	Single	First part of ileum	Submucosa; otherwise not stated	Hemorrhage
34.	Landois ³⁷	1925	25	F	Single	Ileum 50 cm. from ileo-cecal valve	Submucosa mostly but extending into other layers	Abdominal pain, diagnosed appendicitis; no hemorrhage
35.	Bassett ^{*5}	1930	33	M	Single	Jejunum 4 inches from duodenum	Not stated	Hemorrhage
36.	Moore and Schmeisser ⁴⁴	1934	58	M	Single	Third portion of duodenum	Submucosa	Hemorrhage
37.	Miehaëls-son ⁴²	1927	3 mo.	F	Probably single	At junction of middle and lower thirds of ileum and in mesentery	All layers	Pain, hemorrhage and a plum-sized mass in abdomen; diagnosed intussusception
38.	Kortze-born ³⁴	1930	41	M	Single	Ileum 75 cm. from cecum	All layers	Hemorrhage and colicky abdominal pain

* Doubtful case; histologic description not given.

Continued

TYPE II A, DIFFUSE INFILTRATING CAVERNOUS HEMANGIOMA

Treatment	Outcome	Cause of Death	Autopsy	Character of Tumor	
				Gross	Microscopic
Exploratory laparotomy	Not stated			No details given; said to be a cavernous hemangioma	
Resection	Recovery			Lesion 3 to 4 cm. long	Ulceration of mucosa with exposure of bleeding vessel; probably cavernous hemangioma; details not given
Resection	Recovery			Purple mass 8 x 5 x 2 cm., involving entire circumference of intestine and obstructing lumen	Cavernous hemangioma
	Death	Hemorrhage from bowel and extensive pulmonary tuberculosis	Yes	Marked varicosity, 5 cm. in area; two sites of rupture into lumen noted; no other varices; liver and spleen normal	Not reported
	Death	Hemorrhage and obstruction	Yes	Diffuse annular tumor 6 cm. in width, almost obstructing lumen	Large endothelium lined spaces filled with blood
Resection	Death	Post-operative peritonitis	Yes	Lesion 10 cm. long and 3 cm. thick with narrowing of lumen	Cavernous blood-containing spaces of various sizes invaded intestinal wall including mucous and serous coats
Resection	Recovery			Lesion encircled intestine for width of 8 cm.; lumen admitted one finger	Capillary and cavernous hemangioma with former elements predominating

TABLE 1—

NUMBER	AUTHOR	DATE	AGE	SEX	TYPE II A, DIFFUSE INFILTRATING CAVERNOUS HEMANGIOMA			
					Single or Multiple	Portion of Small Intestine	Portion of Wall Involved	Symptoms
39.	Kuhle ³⁵	1932	16	M	Single	Ileum 20 em. below a Meekel's diverticulum	All layers	Hemorrhage
TYPE II B, CIRCUMSCRIBED, OFTEN POLYPOID, CAVERNOUS HEMANGIOMA								
NUMBER	AUTHOR	DATE	AGE	SEX	Single or Multiple	Portion of Small Intestine	Portion of Wall Involved	Symptoms
40.	Kaspar ^{*31}	?	15	M	Single	In a Meekel's diverticulum	Not stated	Hemorrhage
41.	Kaufman ^{*31}	1931	62	M	Single			
42.	Nieoll ⁴⁵	1899	23	F	Single		Submueosa	Intussuseption
43.	Helvestine ²⁵	1923	72	F	Single	Jejunum	Subserosa	None
44.	Merchant ⁴¹	1939	67	F	Single	Jejunum	Submueosa	Sudden shock occurring during convalescence from cholecystectomy
45.	Rankin and Newell, ⁵³ Judd ²⁷	1933 1929	37	F	Single	Duodenum	Not stated; probably submucosa	Repeated gastrointestinal hemorrhages
46.	Ackerman ¹	1937	70	M	Single	Jejunum	Submucosa	

* Quoted from Kaijser; ²⁹ original reference not consulted.

Continued

TYPE II A, DIFFUSE INFILTRATING CAVERNOUS HEMANGIOMA

Treatment	Outcome	Cause of Death	Autopsy	Character of Tumor	
				Gross	Microscopic
Resection	Recovery			Lesion encircled intestine for distance of 5 cm. on one side and 2 cm. on the other; lumen admitted only in index finger	Cavernous hemangioma

TYPE II B, CIRCUMSCRIBED, OFTEN POLYPOID, CAVERNOUS HEMANGIOMA

Treatment	Outcome	Cause of Death	Autopsy	Character of Tumor	
				Gross	Microscopic
Resection	Recovery				Cavernous hemangioma
				Pea-sized tumor in mucosa; <i>intussusception</i>	Hemangioma
Resection	Recovery			Tumor the size of a pigeon's egg, containing 2 small phleboliths, each the size of a split pea; double <i>intussusception</i>	Large thin walled spaces filled with blood
	Death	Pneumonia	Yes	5 x 5 x 4 cm.	Subserosal cavernous hemangioma
None	Death	Peritonitis, intussusception and hemorrhage	Yes	Polypoid, 3 cm. in diameter, <i>intussusception</i>	Cavernous hemangioma
Excision of pyloric cap and tumor	Recovery			Sessile, intraluminal, ulcerating mass 4.5 x 4.0 x 1.5 cm.	Composed of loose connective tissue stroma containing blood spaces and channels of varying size
	Death	Carcinoma of antrum	Yes	Sessile mass 2.5 x 0.5 cm.	Vascular space lined by endothelium

TABLE 1—

NUMBER	AUTHOR	DATE	AGE	SEX	TYPE II B, CIRCUMSCRIBED, OFTEN POLYPOID, CAVERNOUS HEMANGIOMA			
					Single or Multiple	Portion of Small Intestine	Portion of Wall Involved	Symptoms
47.	Klein ³³	1936	40	F	Single	Jejunum	Submucosa, subserosa and muscularis	Abdominal pain; mass size of a fist in lower abdomen
48.	Shillito ⁶⁷	1921	27	M	Single	Ileum	Not stated	Recurrent abdominal pain, vomiting and mass in right lower quadrant, hemorrhage
49.	Aresu ³	1923	40	M	Single	Exact site not stated	All layers	Periodic attacks of intestinal obstruction
50.	Laboulbène ^{*36}	1872	64	M	Single	Doudenum above the papilla of Vater	Submucosa	Hemorrhage and epigastric distress

* Classified under Type III by Kaijser, but the original description places it in this class.

NUMBER	AUTHOR	DATE	AGE	SEX	TYPE III, SIMPLE CAPILLARY HEMANGIOMA			
					Single or Multiple	Portion of Small Intestine	Portion of Wall Involved	Symptoms
51.	Obern-dorfer† ⁴⁶	1929	43	M	Not stated	Upper	Not stated	None
52.	Blahd, Mashke and Karsner ⁸	1923	2 mo.	F	Single	Ileum	All portions except mucosa	Acute intestinal obstruction
53.	Raiford ⁵¹	1932	60	M	Single	Ileum	Submucosa	Hematemesis and obstruction

† Quoted from Kaijser;²⁹ original reference not consulted.

Continued

TYPE II B, CIRCUMSCRIBED, OFTEN POLYPOID, CAVERNOUS HEMANGIOMA

Treatment	Outcome	Cause of Death	Autopsy	Character of Tumor	
				Gross	Microscopic
Resection	Death	Bilateral broncho-pneumonia 9 days post-operative	Yes	Globular, sharply circumscribed mass 4.5 x 4 x 4 cm.; intestinal lumen slightly constricted; some erosion of mucosa	Cavernous hemangioma
Resection	Recovery			No gross description; author suggests that erectile nature of tumor permitted periodic engorgement with resulting obstruction; however intermittent <i>intussusception</i> seems more likely	Cavernous hemangioma
Resection	Recovery			Pedunculated, extending into lumen	Cavernous hemangioma
None	Death	Hemorrhage	Yes	Erectile, "size of an almond", 4 cm. in length, with ulceration of overlying mucosa	Greatly dilated capillary spaces

TYPE III, SIMPLE CAPILLARY HEMANGIOMA

Treatment	Outcome	Cause of Death	Autopsy	Character of Tumor	
				Gross	Microscopic
			?		Capillary hemangioma
Ileostomy	Death	Obstruction	Yes		An invasive and locally malignant capillary hemangioma
Excision	Recovery			Pedunculated, bloody	Tremendously hypertrophied blood vessels with round cell infiltration; simple hemangioma

TABLE 1—

NUM- BER	AUTHOR	DATE	AGE	SEX	TYPE III, SIMPLE CAPILLARY HEMANGIOMA			
					Single or Multiple	Portion of Small Intestine	Portion of Wall Involved	Symptoms
54.	Carbonnel Salazar ^{*14}	1939	9	M	Single	Last part of ileum	Subserosa (?); not clearly stated	Umbilical pain, diagnosed appendicitis
55.	Hanke ²³	1936	15	M	Multiple (3)	Ileum 80 cm. from Rankin's valve	Subserosa	Incidental finding at operation for acute appendicitis
56.	Carman† ¹⁵	1921	22	F	Single	Duodenum	Submucosa	Vomiting and indigestion
57.	Sussig ⁵⁹	1923	3 mo.	F	Multiple (2)	Lower jejunum	Submucosa	Obstruction, vomiting, hemorrhage and intussusception
58.	Hansen	1947	56	F	Single	Lower ileum	Submucosa	Obstruction, intussusception

* This case is classified here without adequate authority for sake of convenience. The author gives no histologic description of the tumor.

† Also reported by Rankin and Newell, by Judd and Rankin, and by Balfour and Henderson.

NUM- BER	AUTHOR	DATE	AGE	SEX	TYPE IV, ANGIOMATOSIS			
					Single or Multiple	Portion of Small Intestine	Portion of Wall Involved	Symptoms
59.	Gascoyen ²¹	1860	44	M			Submucosa and muscularis	None

Continued

TYPE III, SIMPLE CAPILLARY HEMANGIOMA

Treatment	Outcome	Cause of Death	Autopsy	Character of Tumor	
				Gross	Microscopic
Extirpation	Recovery			Size of a lentil; details not given	
Extirpation	Recovery			Spongy, bloody, blue-black, lobulated, size of a walnut	Capillary hemangioma; similar, smaller structures in cecum and ascending colon
Excision	Recovery			5 x 7 cm.	Composed of a mesh of fine capillaries with a few dilated sinuses supported by loose highly cellular stroma
Resection	Recovery			Easily reduced <i>intussusception</i> 10 cm. in length; tumor of papillomatous structure, sessile, size of a walnut; a smaller tumor 2 cm. distally was present; the larger formed apex of <i>intussusciptions</i>	Capillary elements predominated over cavernous elements
Resection	Recovery			Cylindrical mass 9.5 cm. in length and 3 cm. in circumference, projecting into lumen of bowel and causing <i>intussusception</i>	Capillary hemangioma; some ulceration of mucosa

TYPE IV, ANGIOMATOSIS

Treatment	Outcome	Cause of Death	Autopsy	Character of Tumor	
				Gross	Microscopic
	Death	Suffocation from parotid cavernous hemangioma	Yes	Solitary small intestinal tumor originating in submucosa and projecting into lumen; "naevi" in skin, liver and parotid gland	The parotid "naevus" was a cavernous hemangioma

TABLE 1—

NUM- BER	AUTHOR	DATE	AGE	SEX	TYPE IV, ANGIOMATOSIS			
					Single or Multiple	Portion of Small Intestine	Portion of Wall Involved	Symptoms
60.	Raiford ⁵¹	1932	19	F		First portion of duodenum	Not stated	Hemorrhage; "blood tumor" removed from tongue at age of 7 years
61.	Merchant ⁴¹	1939	8	M		Jejunum	Submucosa	Obstruction
62.	Ackerman ¹	1937	81	M		Duodenum, jejunum and ileum	Submucosa, penetrating the muscularis and entering the subserosa	Shock, hemorrhage
63.	McLure and Ellis ⁴⁰	1930	32	F		Duodenum, jejunum, ileum	All layers	Indigestion and hemorrhage
64.	Schuster ⁵³	1937	62	F		First 4 inches of duodenum	Submucosa	Hemorrhage

Continued

TYPE IV, ANGIOMATOSIS					
Treatment	Outcome	Cause of Death	Autopsy	Character of Tumor	
				Gross	Microscopic
Excision	Im- proved				Duodenal tumor composed of connective tissue hypertrophy with many blood filled sinuses
Laparotomy	Death	Gangrene, peritonitis and intussusception	Yes	1.5 x 1.5 cm. beginning to push out into lumen; post-operative <i>intussusception</i> was probably agonal; hemangioma of lip which recurred repeatedly after excision; also hemangiomas of skin and stomach	Cavernous hemangioma
	Death	Rupture of a duodenal subserosal hemangioma retroperitoneally		Multiple tumors in colon, gallbladder, lip and skin	Cavernous hemangiomas in submucosa of the small intestine
Laparotomy; hemangiomas present also in skin, tongue, liver and stomach; biopsy of sections of liver; cavernous hemangioma	Death	Hemorrhage	No	At operation, a site of beginning <i>intussusception</i> was noted	All types of hemangioma seen
	Death	Bronchopneumonia, cardiac failure	Yes	Lesions of "spider" type; some in duodenum were ulcerated; lesions in skin, stomach, lungs and elsewhere; case described by authors as one of familial hemorrhagic telangiectasis	

TABLE 1—

NUMBER	AUTHOR	DATE	AGE	SEX	TYPE IV, ANGIOMATOSIS*			
					Single or Multiple	Portion of Small Intestine	Portion of Wall Involved	Symptoms
65.	Blank† ⁹	1938	4	F			Not stated	
66.	Hansen	1947	66	F		Upper jejunum	Submucoas and muscularis	None

* The cases of Stamm, Ullman, Konjetzny and Jaffé are correctly classified by Kaijser under angiomatosis, but in none of the cases is it specifically stated that the small intestine was involved, although it probably was in most, if not in all of them. The case described by Winternitz and Boggs⁶² is classed by some authors in this group. In reality, however, it was a case of lymphangioma of the intestine with malignant degeneration and unrelated multiple subcutaneous hemangiomas.

† Quoted from Kaijser;²⁹ original reference not consulted.

REPORT OF CASES

Case 1

J. M., a 59 year old white man, was admitted to the Philadelphia General Hospital, January 4, 1940, and died on January 6. His main complaint was anuria of several days' duration. He was too sick to give an adequate history. Physical examination revealed evidence of cardiac and renal failure. The blood urea was 125 mg. per 100 ml. The patient failed steadily and died on the third hospital day.

An unexpected finding at autopsy, performed by Dr. Helen Ingleby, was the presence of "small, firm, rounded nodules consisting of blood clots, in the wall throughout the small intestine". There were also two "thrombosed veins" in the sigmoid. The histologic diagnosis was cavernous hemangioma.

Case 2

A. S., a white woman, 66 years old, was admitted to the Hospital of the University of Pennsylvania on the service of Dr. I. S. Ravdin on January 2, 1940 and died on January 7. She complained of symptoms of respiratory obstruction and hoarseness of three and one-half weeks' duration, associated with a sudden increase in size and hardness of an enlarged thyroid, which she had had for at least two years, but for which she had refused operation. Seven years prior to admission, an adenocarcinoma of the breast had been removed and there had been no evidence of recurrence.

Concluded

TYPE IV, ANGIOMATOSIS

Treatment	Outcome	Cause of Death	Autopsy	Character of Tumor	
				Gross	Microscopic
	Death	In connection with operation on the hemangioma of the thigh	Yes	Hemangiomas of large and small intestine, liver, stomach, thigh and elsewhere	All were cavernous hemangiomas
	Death	Fibrosarcoma of thyroid	Yes	Moderately numerous flat, reddish purple areas of discoloration, easily visible from serosal side of intestine; also had cavernous hemangiomas of liver and in peribronchial lymph node	Cavernous hemangiomas

Physical examination revealed a large, hard thyroid displacing the trachea to the left. Laryngoscopy showed paralysis of the right vocal cord. No skin lesions were noted. An x-ray plate of the chest showed substernal extension of the thyroid. The hemoglobin level was 80 per cent of normal (Tallqvist). The stool was not examined for blood. Tracheotomy was performed preparatory to x-ray treatment but the trachea suddenly became obstructed and the patient died on the sixth hospital day.

Autopsy, performed by Dr. Harold Horack, revealed fibrosarcoma of the thyroid with partial necrosis, secondary fibrosarcoma of the kidneys, hemangioma of the liver, submucosal (sic) hemangioma of the jejunum and hemangioma and lymphangioma of a lymph node. The description of the jejunum was as follows: "Beneath the serosal (sic) surface of the upper jejunum there are moderately numerous flat, reddish purple areas of discoloration which on section appear vascular and contain dark venous blood. Except for these lesions, the remainder of the small bowel and the large bowel, rectum and appendix are normal. The mesentery is entirely normal."

Case 3

M. W., a 56 year old Negress, was admitted to the gastro-intestinal section of the Hospital of the University of Pennsylvania, February 25, 1941, on the referral of Dr. Harold E. Farmer of Wayne, Pennsylvania. Her present complaint was abdominal distress of one year's duration. One year before entry, the patient had had an attack of mild epigastric pain, made worse by food but controlled by powders. The attack subsided after the patient was put on a low fat diet. Five months, and again one month, prior to entry, the patient had had attacks of cramplike upper abdominal distress not associated with food, defecation, breathing, or position but made worse by cathartics which the patient took frequently for constipation. Belching and passing of flatus occasionally relieved the distress. There was no true fat intolerance. The stools were normal in color and no blood had ever been noted. The patient had suffered from "nervousness" since the onset of her menopause

eight years previously. She had had a right femoral hernia for more than twenty years with no signs or symptoms other than the visible and palpable swelling. She had lost more than 20 pounds in the past year. She had had measles in childhood. Her family history, history by systems and her habits were negative. There was no known family history of benign or malignant neoplasm.

Physical examination on the day of entry into the clinic, showed a well developed and well nourished Negress with evidence of recent weight loss. The patient was very tense with marked tremor of the fingers but moist cool palms. The temperature was 99.2 F. orally, blood pressure 146/100, and her pulse rate 100. The skin and peripheral arterics were normal and there was no lymphadenopathy. Examination of the eyes including the eye grounds was normal. The teeth were carious but the tongue and pharynx were negative. There was an apparent fullness in the neck in the region of the thyroid but there was no definite palpable thyroid enlargement and there was no bruit. The heart was normal in size but presented a rather loud apical systolic murmur which was best heard in the left lateral position and was accentuated by exercise. There was no diastolic murmur. Examination of the abdomen revealed generalized voluntary rigidity without tenderness and a right femoral hernia one-half the size of an egg. Varicosities were present over both lower legs. A chemical examination of the stool was positive for blood. Fluoroscopic examination of the chest was negative. Rectal examination was negative.

No definite diagnosis was made at this time but it was felt desirable to rule out hyperthyroidism, rheumatic heart disease and hidden neoplasm. The patient was put on mineral oil and belladonna and instructed to stop taking cathartics.

On March 6, she returned to the clinic for determination of the basal metabolic rate. Since her previous visit she had had intermittent upper abdominal cramps, culminating the day before her visit in very severe epigastric pain for which she induced vomiting without relief. The pain gradually subsided without treatment, leaving only slight nausea.

Physical examination on March 6 showed a temperature of 99.8 F., pulse rate of 104 and a respiratory rate of 16. Abdominal findings were unchanged. On the following day the patient was admitted to the Hospital of the University of Pennsylvania on the service of Dr. O. H. Perry Pepper. She had had nearly continuous upper abdominal cramps for twenty-four hours, and had vomited spontaneously twelve hours previously. On the morning of the second hospital day the patient vomited fecal material. On auscultation loud high-pitched peristaltic sounds appearing in rushes could be heard all over the slightly distended abdomen. No masses could be felt. A diagnosis of intestinal obstruction and femoral hernia was made. It was felt that the femoral hernia was incidental and that abdominal exploration was indicated. X-ray studies on March 10 showed normal intrathoracic contents. There was no evidence of free gas in the abdomen. Many dilated loops of small intestine were seen which in the erect position formed a semistepladder appearance with fluid levels. Since there was no evidence of more than a very small amount of gas in the colon, the obstruction was thought to be in the distal end of the small intestine. Blood studies on February 20 had shown an erythrocyte count of 4.8 million, leukocytes 7000, and hemoglobin 95 per cent. A blood smear was normal with 75 per cent polymorphonuclear leukocytes, 10 per cent lymphocytes and 15 per cent monocytes. On March 8, the erythrocyte count was 5.3 million, with leukocytes 7000, and hemoglobin 105 per cent. The cell volume was 49 per cent. The corrected sedimentation rate (Wintrobe) was 36 mm. in one hour. On the same day, the blood urea nitrogen was 18 mg. per 100 ml., the fasting blood sugar was 112 mg. per 100 ml., the blood chlorides 94.7 M Eq. The van den Bergh test showed a delayed direct reaction and an indirect reaction of 0.6 unit. Repeated urinalyses were negative. Kolmer and Kahn tests were negative on two occasions.

The patient was transferred to the service of Dr. E. L. Eliason and, on March 12, after a Miller-Abbott tube had been passed, an operation was performed under spinal anesthesia, through a lower midline incision. The report of the operation follows: "Dilated 'fighting loops' (restrained only with difficulty from escaping from the abdominal cavity at laparotomy) of small intestine were found which contained the Miller-Abbott tube. Two short



FIG. 1. Case 3. Ileum opened to show tumor in place. The intussusception has been reduced.

FIG. 2. Case 3. Capillary hemangioma of lower ileum. X 160.

intussusceptions were found and reduced. But in the lower ileum, a mass other than the tube was felt which seemed long and polypoid. This was obviously the point of the patient's partial obstruction as the intestine proximal to the lesion was hypertrophied, dilated and 'fighting' while that distal was more normal in appearance. On the serosal surface of the ileum at the site of the lesion was an umbilicated puckered area with an edematous polypoid tab projecting at the mesenteric border. The intussusciptions were somewhat edematous and contained petechial hemorrhages. The exact nature of this mass could not be determined by gross examination but the indication for resection seemed to be clear. At the start of the operation the Miller-Abbott tube, which had passed the lesion and was in the ascending colon, had been withdrawn to a site proximal to the lesion. The lesion was resected and a side-to-side anastomosis performed between the distal and proximal segments of the ileum. No attempt was made to repair the femoral hernia. The patient was returned to the ward in good condition."

The postoperative course was smooth and uneventful and she was discharged as cured on March 31. Two months after her operation she still complained of slight nervousness but had gained 8 pounds.

The report of the pathologist, Dr. A. E. Bothe, came as something of a surprise; "Specimen consists of about 20 cm. of ileum which has been opened (Fig. 1). In the center of the mucosal surface there projects a cylindrical tumor-like mass which is 9.5 cm. in length and 3 cm. in circumference at its attachment. The distal half of the mass is dark gray-green, rough and friable and the mucosal surface is ulcerated at several points. The proximal half is covered by granular red-brown mucosa. The bowel itself has prominent mucosal folds and these and the muscles are hypertrophied at one end. On the outside opposite the attachment of the mass there is a dimple which leads downward into a funnel-like pit. This apparently stops after about 1 cm. and is not continued into the mass. Near the dimple is a rounded soft polypoid mass of tissue, 1 cm. in circumference, somewhat resembling an appendix epiploica. It is gray in color and when cut is found to consist of homogeneous gray moderately soft tissue with a small smooth eccentric cavity (2 x 5 mm.). This cavity is not connected externally but leads downward into a smooth tract 1 mm. in diameter. A probe can be passed into the center of the polypoid mass for half its length.

"Section of the polypoid mass shows the outer surface to be gray and beneath it the tissue is in part solid, dark gray and in part red and hemorrhagic."

Histologic examination revealed the mass to be a capillary hemangioma with many small and a few moderately dilated capillary spaces in a moderately abundant cellular myxomatous stroma (Fig. 2).

Acknowledgments. At the time the material was collected for this paper (1940-41), the author was Henrietta Heckscher Research Fellow in Medicine at the Hospital of the University of Pennsylvania on the service of Dr. O. H. Perry Pepper. He wishes to acknowledge his gratitude to Dr. Pepper and to the late Dr. W. Osler Abbott for their assistance, stimulation and encouragement. The author is also indebted to Dr. Edward B. Krumbhaar for assistance in searching the records of the Philadelphia General Hospital for cases of hemangioma of the small intestine.

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ELEVATED SERUM AMYLASE IN ALCOHOLICS*

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Accumulated evidence leaves little doubt that alcohol may play a significant role in the production of pancreatitis (pancreatic necrosis). The literature on pancreatitis includes a number of reports^{2, 5, 9, 10, 12} of sudden death following the ingestion of large amounts of alcohol, with the finding at autopsy of acute hemorrhagic pancreatitis. Egdahl⁵ analyzed 105 cases of acute hemorrhagic pancreatitis and attributed 17 of them to the effect of alcohol. McWhorter,⁹ in his series of 64 patients with acute hemorrhagic pancreatitis, found that among those who were alcoholics, the mortality rate (70 per cent) was second only to the mortality rate among those who were very obese. Myers and Keefer¹⁰ found a rough correlation between the duration of alcoholism and the degree of pancreatic damage in 6 patients. Acute hemorrhagic pancreatitis was the sole cause of death in patients with a relatively brief alcoholic history, but in a patient who had been drinking for many years, there was in addition, a chronic sclerosing pancreatitis. Weiner and Tennant¹² reported two cases of fulminating pancreatitis following the ingestion of alcohol and reviewed 4000 autopsies. They found that of 51 patients dying during acute alcoholic episodes, 53 per cent had pancreatic lesions, almost all of which were acute. This was fifty times the incidence of acute pancreatitis in the general autopsy series. Clark² presented a study of 36 cases of pancreatic disease in severe alcoholics. Acute hemorrhagic pancreatitis was held responsible for the deaths of 15 patients in the series (in 14, death occurred during or shortly after an alcoholic bout). Acute lesions were found in 19, mixed acute and chronic lesions in 5, and purely chronic lesions in 12 of the 36 cases. Gross and Guleke⁸ stated that from a study of the literature and from their own experience it was evident that anatomic alterations of the pancreas, in the form of an increase in the intralobular and interlobular fibrous tissue, were common in chronic alcoholics. In their classic monograph on chronic relapsing pancreatitis, Comfort, Gambill and Baggenstoss³ were able to evaluate the role of alcohol in 25 of 29 patients. A history of the use of alcohol was obtained in 68 per cent of these patients, and of heavy alcoholism in 32 per cent. In 4 patients alcoholic excess was definitely incriminated as the precipitating agent in typical stormy exacerbations of the disease. Weiner and Tennant¹³ found chronic pancreatitis in 2.5 per cent (97) of 4000 of their autopsies, whereas it occurred in 47 per cent of the chronic alcoholics in the series, or approximately nineteen times as often as in the nonalcoholics.

A recent report from this hospital⁴ described two cases of chronic pancreatitis with diffuse parenchymal calcification; both patients were chronic alcoholics. Carter,² in 1945, drew attention to the fact that an acute surgical abdominal condition in an alcoholic is often simulated by acute interstitial pancreatitis, a

* Received for publication, September 13, 1947.

TABLE 1
DETAILS CONCERNING ALCOHOLISM, PRESENCE OF SIGNS OR SYMPTOMS
SUGGESTING PANCREATITIS AND SERUM AMYLASE VALUES IN 50 CHRONIC
ALCOHOLICS, WITH CONTROL VALUES FOR SERUM AMYLASE

PATIENT NUMBER	HISTORY OF ALCOHOL- ISM	DURATION OF RECENT BOU	BEVERAGE: W, WHISKEY; B, BEER	NAUSEA AND/OR VOMITING	PAIN IN ABDOMEN OR BACK	TENDERNESS IN EPIGASTRIUM OR COSTOVER- TEBRAL ANGLE	HYDROLYSIS OF SERUM AMYLASE OF PATIENT	HYDROLYSIS OF SERUM AMYLASE OF CONTROL
	<i>years</i>						<i>per cent</i>	<i>per cent</i>
1	25	1 wk.	W-B	+	+	+	51*	28
2	20	4 da.	W-B	0	0	0	33	28
3	15	1 wk.	B	0	0	0	37*	30
4	3	1 wk.	W	0	0	0	54*	13
5	10	17 da.	W	+	0	0	37*	25
6	15	1 mo.	W	0	0	0	47*	25
7	10	2 wk.	W-B	0	0	0	30	29
8	25	10 da.	Wine	0	0	0	22	18
9	30	3 wk.	W	+	0	0	15	10
10	19	1 wk.	W-B	0	0	+	34	25
11	20	4 mo.	W	0	0	0	41*	26
12	18	3 mo.	B	+	+	++	28	24
13	20	1 wk.	W-B	+	0	0	17	19
14	6	2 wk.	W-B	0	+	0	11	28
15	10	1 wk.	W	0	0	0	42*	22
16	5	3 mo.	W-B	+	+	++	45*	26
17	10	5 da.	B	0	0	0	18	34
18	20	2 wk.	W-B	+	+	0	22	44**
19	10	1 mo.	W	0	+	0	20	34
20	25	1 wk.	W	0	+	+++	19	25
21	25	2 wk.	W	0	0	0	34	26
22	7	3 wk.	B	+	+	+	28	25
23	8	2 wk.	W-B	++	+	+	34	28
24	1	3 wk.	W	+	0	0	27	14
25	3	2 da.	W	0	0	0	45*	22
26	15	4 mo.	W	0	0	0	17	22
27	19	3 mo.	W	+	0	0	35	21
28	10	4 mo.	W	+	+	0	23	22
29	4	4 da.	W	+	0	0	23	28
30	20	6 mo.	Saki	+	0	0	35	30
31	2	5 mo.	W	+	0	0	35	30
32	2	2 mo.	W-B	0	0	0	25	24
33	2	2 wk.	W-B	+	+	0	35	25
34	8	6 mo.	W-B	0	0	0	21	25
35	15	1 wk.	W	+	0	0	34	31
36	25	1 mo.	B	+	0	0	23	34
37	6	10 da.	W-B	0	0	0	16	32
38	10	2 wk.	W-B	0	+	+	27	25
39	25	1 wk.	W-B	0	0	0	26	22
40	10	6 wk.	W-B	0	0	0	21	26

* Indicates elevated value of amylase; ** indicates elevated value of amylase in control.

TABLE 1—*Concluded*

PATIENT NUMBER	HISTORY OF ALCOHOLISM	DURATION OF RECENT BOUT	BEVERAGE: W, WHISKEY; B, BEER	NAUSEA AND/OR VOMITING	PAIN IN ABDOMEN OR BACK	TENDERNESS IN EPIGASTRIUM OR COSTOVERTEBRAL ANGLE	HYDROLYSIS OF SERUM AMYLASE OF PATIENT	HYDROLYSIS OF SERUM AMYLASE OF CONTROL
	<i>years</i>						<i>per cent</i>	<i>per cent</i>
41	8	4 da.	B	0	+	0	30	30
42	27	2 da.	B	0	0	0	27	30
43	9	1 wk.	W	+	+	+	29	26
44	16	1 wk.	W	0	0	0	43*	12
45	6	2 wk.	W	+	+	+	21	27
46	4	6 wk.	W-B	0	0	0	15	21
47	5	1 mo.	W	0	0	0	46*	31
48	18	14 da.	W	0	+	0	40*	33
49	7	2 yr.	W	+	+	+	26	28
50	5	4 wk.	W-B	+	+	+	30	31

condition for which surgery is not indicated. He presented 11 such instances in which the patients being alcoholic had a high level of serum amylase: in four of the patients operation disclosed a tense, edematous pancreas, while the remaining patients were not operated on and recovered uneventfully.

Following Carter's report, the possibility of *subclinical* pancreatitis in alcoholics suggested itself. It seemed logical to suppose that if alcohol could produce interstitial pancreatitis severe enough to be mistaken for a perforated peptic ulcer, it could also produce milder degrees of inflammation which might be misdiagnosed as "alcoholic gastritis", or go entirely unnoticed clinically. To study this possibility, amylase determinations, using the Fennel⁶ method, were made on the serum of 50 alcoholics and of 50 nonalcoholic control patients on the Neuropsychiatric Wards of The Queen's Hospital. This test was performed by adding 0.1 cc. of a 1:10 serum dilution to a substrate of starch solution, incubating for thirty minutes, adding a constant quantity of iodine solution to produce a blue color and measuring the density of the color against that of the substrate after iodine only had been added. The results were read as per cent of starch hydrolyzed. The upper limit of normal was 35 per cent hydrolysis.

Blood was drawn from each patient as soon as possible after admission, and information was sought as to the kind and amount of beverage consumed, the duration of alcoholism, the duration of the most recent drinking bout and the time elapsed since the last drink of alcohol. Details of symptoms suggestive of pancreatitis were recorded and then each patient was particularly examined for epigastric and costovertebral angle tenderness, which might suggest pancreatitis. The findings are tabulated in the accompanying table. Unfortunately, similar examinations on the nonalcoholic patients used as controls were not feasible because most of them were acutely psychotic.

The principal finding was definite elevation of serum amylase in 12 (24 per cent) of the alcoholics. Of these, 3 were slightly elevated (from 36 to 40 per

cent hydrolysis), 6 were moderately elevated (from 41 to 45 per cent hydrolysis) and 3 were markedly elevated (more than 45 per cent hydrolysis). In comparison, only 1 (2 per cent) of the controls had a high value. This patient was a paranoid schizophrenic who complained of periumbilical pain. Before we could determine whether this pain was real or delusional, and whether his serum amylase remained elevated or not, he was removed from the hospital against advice.

The average duration of alcoholism in the 12 patients with elevated serum amylase was eleven years, exactly the same as in the unaffected alcoholics. Nor is it possible to incriminate a particular kind of alcoholic beverage. The interval of time between the last drink and the test for serum amylase may be critical in a study such as this because many previous investigators have shown that amylase remains elevated in the serum for only a short period, even in acute hemorrhagic pancreatitis. Serum was obtained less than forty-eight hours after the last drink in all but 5 patients (Numbers 24, 30, 32, 40, 45), and in none of these 5 patients was there an elevated amylase.

DISCUSSION

Exactly how alcohol produces pancreatitis is not known. Egdahl⁵ considered that the pancreatitis was the result of the acute gastroduodenitis produced by the alcohol. It is conceivable that sudden vascular congestion and tiny capillary hemorrhages might be produced in the pancreas with resulting acinar damage and liberation of trypsin. Once trypsin is extravasated, the rapid chain of events characteristic of acute hemorrhagic pancreatitis occurs: digestion of tissue, necrosis of blood vessel walls, rupture, hemorrhage, disruption of more acini and liberation of more trypsin and lipase, with fat necrosis. Should the capillary hemorrhage fail to damage enough acini to produce a severe process, a small area of fibroblastic proliferation and eventually of scar tissue might be the only result. If this process were repeated many times in a confirmed alcoholic, fibrosis or possibly calcification of the pancreas might result.

Clark⁴ stressed the role of inspissated pancreatic secretions causing obstruction of the ducts in 30 patients in his series of 36 alcoholics with pancreatitis. Rupture of some of the smaller pancreatic ducts due to obstruction was cited as the mechanism responsible for acinar damage and for liberation of trypsin.

Rich and Duff¹² were much impressed with obstruction produced by metaplastic duct epithelium in their study of pancreatitis. They also postulated that alcohol might stimulate the production of trypsin exactly as food does, and thus magnify the destructive action of pancreatic juice once it is liberated from its normal channels. Their chief contribution, however, was the demonstration that liberated trypsin is sufficiently irritating to cause destruction of tissues and necrosis of blood vessel walls with resultant rupture and hemorrhage. This fact serves as the single common denominator in all the theories cited above as to how alcohol produces pancreatitis.

The present report does not throw any further light on the pathogenesis of "alcoholic pancreatitis", but does suggest that the range of recognized effects of

alcohol on the pancreas should be extended to include cases of subclinical acute pancreatitis.

SUMMARY

Elevated serum amylase was found in 12 (24 per cent) of 50 chronic alcoholics following recent alcoholic intoxication, but in only 1 of 50 control patients. It is suggested that elevation of the serum amylase in alcoholics represents evidence of subclinical pancreatitis and that this condition may be a precursor of more severe clinical forms of pancreatitis.

Acknowledgment. We wish to express our sincere appreciation to Betty Jane Early, M.T., who performed all of the amylase tests on which this study is based.

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DIFFUSE INTERSTITIAL MYOCARDITIS IN A CASE OF EPIDEMIC ENCEPHALITIS*

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Myocardial involvement is scarcely mentioned in the vast literature on epidemic encephalitis. V. Economo⁶ remarked, in 1929, that "myodegeneratio" may develop in severe instances of encephalitis. Tilney and Howe,²⁷ reporting on one patient after an illness of two weeks, observed the myocardium to be "pale and very flabby" without areas of fibrosis or fatty change; but no histologic examination was given in their report. Other reports on changes in the heart include observations on diffuse edematous thickening of the mitral and aortic valves¹ and pericarditis in a case of acute epidemic encephalitis where the serous exudate contained a considerable number of lymphocytes.¹² The myocardium, however, is not considered in most of the monographs on the pathology of encephalitis;^{4, 9, 18, 19, 23-26, 28} nor is encephalitis of viral origin mentioned among the causes of myocarditis in monographs on heart diseases.^{13, 16, 22, 30, 31}

Diffuse, interstitial, nonpurulent myocarditis was observed by me in a sporadic case of acute epidemic encephalitis. A report of this case appears pertinent in the light of the interest in myocarditis of viral origin displayed in the recent literature.

REPORT OF CASE

Clinical Data

History. An Armenian male, 65 years of age, was admitted to the Hadassah University Hospital in Jerusalem in a semistuporous condition which was said to have developed within a period of eight days. The illness began quite suddenly with a temperature of about 39 C. (102.2 F.), which had continued since that time. The past history was non-contributory. On admission, respiration was of the Cheyne-Stokes type with long intervals of apnea. The skin was slightly cyanotic. Physical examination revealed no abnormalities of the oral cavity, the heart, lungs or abdominal organs. The pulse rate was 100 per minute, the blood pressure 100/60. There was ptosis of the left eyelid and left divergent strabismus. The pupils were narrow and reacted slowly to light. The ocular fundus could not be examined because of residual changes following trachoma. The facial nerve was unaffected on either side. The extremities were not paralyzed; a slight spasticity was suspected in the upper extremities. Patellar and heel reflexes were normal and there were no pathologic reflexes. Urinary incontinence was noted.

Laboratory findings. The cerebrospinal fluid was under normal pressure; the protein value was 30 mg., glucose 112 mg., and no cells were present. The gold sol test was negative and bacteriologic cultures remained sterile. The blood revealed a leukocytosis of from 11,000 to 24,000. The differential count remained fairly constant and showed a marked lymphocytosis. The urea and chloride levels in the blood were normal and glucose was 167 mg. per 100 ml. The urinalysis was negative. The blood Wassermann, Kahn, Widal and Weil-Felix tests were repeatedly negative.

* Received for publication, September 19, 1947.

Subsequent course. Therapy consisted of penicillin and 4 Gm. of sulfapyridine daily. The patient's condition became rapidly worse; stupor deepened and respiration became more difficult. The leukocyte count rose to 24,000 on the day before death. The rectal temperatures fluctuated between 39.2 and 37.6 C. (102.6 to 99.7 F.) and the pulse rate varied between 115 and 140 per minute. The cerebrospinal fluid revealed no pathologic findings until the day before death, at which time there were still no cells, but the protein value was 100 mg. and glucose was raised to 155 mg. The Wassermann, Kahn and gold sol tests were negative. The patient died six days following admission, on the fourteenth day of his illness.

Final clinical diagnosis. Nonpurulent encephalitis.

POSTMORTEM FINDINGS

The *anatomic diagnoses* were: Nonpurulent epidemic encephalitis; internal hydrocephalus, moderate; marked hyperemia of the brain and pia-arachnoid; nonpurulent interstitial myocarditis; splenic hypertrophy (weight 290 Gm.) of the chronic septic type; old echinococcus cyst of the spigelian lobe of the liver; nodular adenomatoid hypertrophy of the prostate gland; muscular hypertrophy (slight) and solitary false diverticulum of the urinary bladder; slight hydropelvis of the kidneys.

Pertinent macroscopic findings. Necropsy was performed fourteen hours after death. The body showed a great deal of wasting. The pertinent findings were as follows. The *dura* was slightly tense. The *sinuses* contained dark red blood and soft clots, with the exception of the right cavernous sinus which contained a few friable clots of grayish red color. The *pia-arachnoid* was smooth and glistening; in the parietal regions it was elevated by clear fluid. Hyperemia of the pia-arachnoidal vessels was conspicuous. The basal arteries of the brain were without abnormalities. The *brain* was somewhat soft in consistency. The lateral ventricles were moderately dilated, their lining was smooth and the lumens contained an increased amount of clear colorless fluid. Sections of the brain substance were moist, with extremely marked hyperemia throughout. The *orbital cavities* and *middle ears* showed no lesions. The *heart* weighed 260 Gm. The pericardium was smooth and glistening. The ventricles were contracted. The endocardium and valves were without gross changes, with the exception of the semilunar valves of the aorta, which were slightly thickened near the commissures. The myocardium was of a dark reddish color which, in the wall of the left ventricle (especially in its lower portion and in the septum), was variegated by many parallel, grayish streaks. No gross scarring was seen in numerous sections. The coronary arteries showed only a few yellowish intimal plaques; the intima of the anterior descending branch included minute calcified plaques. The *aorta* and its large branches had elastic walls. The intima of the aorta presented only scattered atheromatous plaques.

HISTOLOGIC FINDINGS

Brain. Sections from the thalamus, pons and the cephalic portion of the medulla oblongata showed inflammatory lesions of varying intensity. The small and medium-sized blood vessels were markedly engorged with blood. Perivascular accumulations of cells were seen in all sections examined; while in several blocks some of the vessels were spared by this process, in the vicinity of the aqueduct and of the nuclei olivares all the blood vessels were affected. The infiltrations were composed of lymphocytes and of larger cells having the appearance of histiocytes (Fig. 1). In sections taken from the pons and the thalamus, amyloid bodies were found in abundance, mainly near blood vessels (Fig. 2). The glial nuclei were evenly distributed. Only two isolated instances of neuronophagia were found, in sections through the optic thalamus (Fig. 3).

Myocardium. Blocks were taken for embedding in paraffin from the wall of

the left ventricle, from the apical portion of the interventricular septum, as well as from the "six strategic areas" for the investigation of rheumatic lesions.¹⁰ Furthermore, frozen sections were prepared from different parts of the cardiac wall. Muscle fibers showed normal striation and brown pigmentation in slight to moderate degree. Fatty change of the muscle could not be demonstrated. Extra- and intramuscular branches of the coronary arteries showed no evidence of sclerotic lesions, except for discrete lipid deposits in the intima of some intramuscular arteries. In a few areas the periarterial connective tissue appeared slightly more abundant than normal, and it contained small groups of lymphoid cells at only a few points. There were discrete calcified deposits in the mitral ring near the upper border of the muscular septum and at the apex of the posterior papillary muscle of the mitral valve.

Sections from all areas contained accumulations of cells which were mainly composed of small lymphocytes and occasionally also included a limited number of polymorphonuclear leukocytes (Fig. 4). These foci, as a rule, followed the course of muscle fibers for a short distance, pushing the fibers slightly aside without causing any visible injury. Cellular foci of this kind were present in greatest frequency in sections of the posterior papillary muscle of the mitral valve; some were discovered at different points in the wall of the left ventricle; while only a few infiltrations were seen in sections of the pulmonary conus and within the interventricular septum. The horizontal portion and the left branch of the atrioventricular bundle were found to be free from pathologic change. Small accumulations of lymphoid cells were also present in the subepicardial fat and in the adventitia of a medium-sized arterial branch, in a block taken from the pulmonary conus. Blocks including one section through each of the cardiac valves revealed no evidence of acute or prior lesions such as vascularization or fibro-elastic hypertrophy.

ANIMAL EXPERIMENTS

The following animal experiments were performed by Dr. H. Bernkopf of the Department of Hygiene and Bacteriology of the Hebrew University. Tissues from aqueductal, thalamic and different cortical areas of the brain were combined and minced and a 10 per cent suspension in physiologic saline was prepared. This suspension was inoculated intracerebrally and intraperitoneally into eight mice. All animals remained well during an observation period of one month.

DISCUSSION

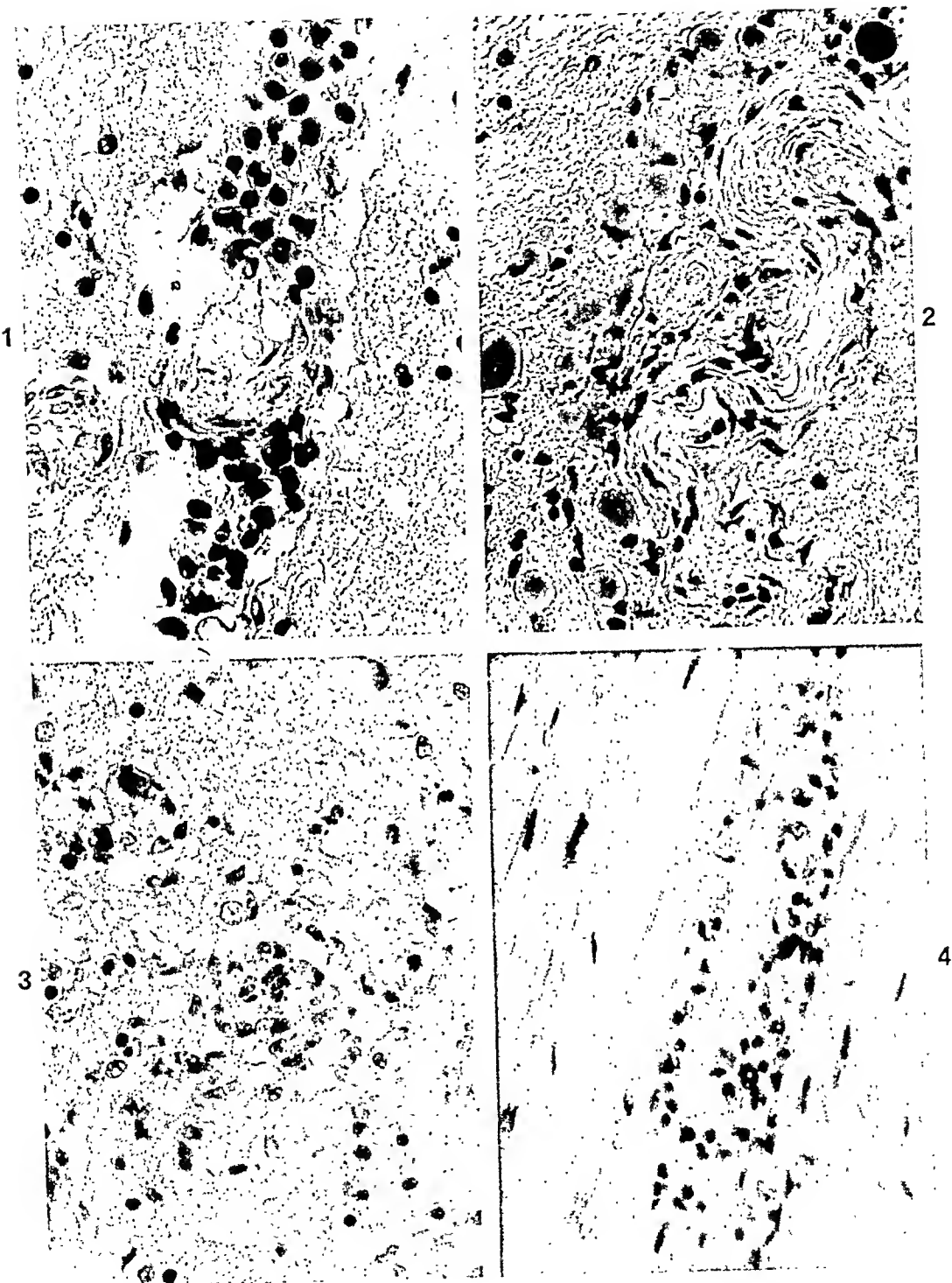
Histologic study of the brain revealed perivascular infiltrations of a character and a distribution held to be characteristic of acute epidemic encephalitis. The almost complete absence of lesions in the medulla oblongata, the rarity of neuronophagia and the entire absence of polymorphonuclear leukocytes within the

FIG. 1. Perivascular accumulations of round cells in brain. $\times 540$.

FIG. 2. Amyloid bodies surrounding an artery in the optic thalamus. $\times 420$.

FIG. 3. Satellitosis and neuronophagia in the optic thalamus. $\times 420$.

FIG. 4. Focus of lymphocytic infiltration in the myocardium. $\times 550$.



FIGS. 1-4

infiltrations, should be taken into account in a differential diagnosis with poli-encephalitis.^{3, 11, 23} Moreover, the negative result of bacterial culture of the blood and the negative outcome of the inoculation into mice of brain taken at necropsy, help to exclude other types of encephalitis.

In the heart, myocardial infiltrations of small round cells were the only pathologic change to be observed. In spite of the advanced age of the patient the coronary arteries showed only a minimum of intimal hyperplasia which caused no narrowing of the lumen at any point. No scarring was found in any of the sections examined, and the valves were free of acute or chronic lesions.

The type and mode of extension of the cellular infiltrations in the heart, revealed no features characteristic of rheumatic lesions. A syphilitic etiology was also extremely unlikely in view of the negative serologic tests with blood and cerebrospinal fluid, and of the entirely negative findings in the aorta, the aortic ostium and the cerebral blood vessels. The patient received medication with sulfapyridine for a period of six days; but the histologic changes in our case showed no resemblance to those observed following treatment with sulfonamide preparations which are distinguished by the polymorphonuclear character of the cells, the presence of abundant eosinophils and a definite affinity of the lesion for blood vessels.^{8, 17}

It appears, therefore, that other than the encephalitis, there were no pathologic changes of obvious significance associated with the myocarditis. At the present stage of knowledge a relationship between these two lesions cannot be conclusively demonstrated, and one may not deny the possible diagnosis of "acute isolated myocarditis" appearing by simple coincidence in a case of epidemic encephalitis. Nevertheless, a viral myocarditis is not unlikely, in view of a growing list of viral diseases in which myocarditis has been shown to occur, and of histologic features which are similar to those observed by us. Saphir,²⁰ in his comprehensive review, mentioned the fact that myocarditis occurs in typhus, Rocky Mountain spotted fever, and yellow fever. There are further reports on myocarditis in measles,⁵ poliomyelitis,^{20, 21} and in influenza A infections.⁷ From electrocardiographic studies, myocardial changes also have been suspected in infectious mononucleosis^{14, 29} and in infectious hepatitis.^{2, 15}

All these observations have been made on diseases which have been well described in textbooks and articles for many years, and in which the myocardial lesion has escaped attention until quite recently. Several of the authors cited have pointed out that this may have been due to the comparative neglect with which the myocardium, in the absence of gross changes, is treated in the accepted routine necropsy procedures. Several generations of pathologists have been trained to take blocks of the myocardium routinely through the central portion of the wall of the left ventricle for histologic sections. With regard to rheumatic fever, it was shown some time ago that the chances for observation of the specific lesion are lower in the traditional blocks of tissue than in certain other areas of the heart.¹⁰ The same is true for other types of myocarditis which may attack only limited portions of the myocardium, albeit with fatal results.^{20, 31}

In cases of epidemic encephalitis clinical observers have occasionally recorded

sudden stoppage of the heart or rhythmic irregularities which were interpreted as being caused by nervous disturbances of central origin.^{6, 9, 15} It is not unlikely that more extended histologic studies will prove that a certain number of such instances are due to organic cardiac lesions.

SUMMARY

Interstitial, nonpurulent myocarditis was observed at necropsy in a case of acute epidemic encephalitis. The possibility of a relationship between both lesions is discussed.

Acknowledgment. The author is indebted to Dr. M. Rachmilewitz for permission to abstract the clinical history of the case.

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A CASE OF *SHIGELLA ALKALESCENS* CYSTOPYELITIS AND BACTEREMIA*

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While the pathogenicity of *Shigella dysenteriae*, *Shigella ambigua*, *Shigella paradysenteriae* and *Shigella sonnei* is above question, it took the concentrated efforts of several workers to prove that *Shigella alkalescens* also must be classified among the organisms which are able to produce human disease. The publications of Neter,¹⁰ Felsen and Wolarsky,⁴ Ingram and Heimann,⁵ and Lieberman⁷ and the autopsy findings of de Assis *et al.*² and Rigdon *et al.*¹¹ gave definite proof of the pathogenic power of *Sh. alkalescens*.

Although *Sh. alkalescens* is often isolated from the intestinal tract, we were unable to find any literature reporting the recovery of this type of *Shigella* from urine or blood. Pyelitis due to shigellae is not observed often. Neter⁹ collected 14 cases from the literature and added 3 of his own. Haynes *et al.*⁵ reported 4 cases. It seems that pyelitis caused by shigellae occurs more frequently in pregnancy than in any other condition. Bacteremia due to shigellae is rarely seen. Among the cases reported from reliable sources, Ashworth and Upchurch¹ surveyed 24 such instances and Dodd and Swanson³ described three cases. *Shigella* bacteremia is more likely to develop in children than in adults and in cachectic patients rather than in healthy individuals.

The scarcity of *Shigella* infections of the urinary tract and of *Shigella* septicemia, together with the hitherto unobserved occurrence of *Sh. alkalescens* in urine and blood simultaneously, prompted report of the following case.

REPORT OF CASE

A 22 year old nurse was admitted to the hospital on April 9, 1947, with the history of frequency and burning on urination for two weeks, and generalized abdominal cramps and diarrhea with watery stools without blood for two days. At the time of hospitalization the pain was worse in the right lower quadrant and the patient had a chill. There was no diarrhea on this day or subsequently during her stay in the hospital. On examination she appeared to be acutely ill. The temperature was 102 F. rectally, the pulse rate 110 per minute, the rhythm regular, and the respiratory rate 20 per minute. There was slight tenderness on deep palpation in the right lower abdominal quadrant, but no rigidity. Tenderness on percussion was present in the right costovertebral angle. The rest of the physical examination, including pelvic and rectal examinations, were negative.

A catheterized urine specimen contained 35 pus cells and 15 red blood cells per high power field. The blood hemoglobin was 81 per cent; the red blood cell

* Received for publication, October 1, 1947.

count was 3,980,000 and the white blood cell count 5900 on admission, and 7800 on the following day. The differential counts were essentially normal. The provisional diagnosis was acute cystopyelitis. Cultures of the urine and blood were obtained and the patient was placed on sulfadiazine and sodium bicarbonate 1 Gm. each q.i.d.

On April 12, 1947, three days after admission, *Sh. alcalescens* was identified in the patient's blood and urine. The body temperature dropped to normal on April 10, rose to 100 F. on April 11, 99.4 F. on April 12, and 99.2 F. on April 13, and remained normal thereafter. A blood sulfa level obtained on April 15 was 81 mg. per 100 ml. On this day, the white blood cell count was 4100 with 5 per cent band forms, 51 per cent neutrophilic polymorphonuclears, 2 eosinophils, 2 basophils, 34 small lymphocytes and 6 monocytes.

X-ray films of the colon on April 11, 1947 (Dr. J. Arendt), revealed a fine degree of roughening of the mucosal pattern extending from the transverse to the descending colon and sigmoid, not sufficient to be considered abnormal. Flat film of the abdomen did not reveal abnormalities. There was a mild vaginal discharge containing a moderate number of pus cells, epithelial cells and lactobacilli. Urethral and cervical smears were negative for neisseriae. The catheterized urine specimen contained from 15 to 20 pus cells per high power field on April 12, and from 5 to 10 cells on April 14. Subsequent samples examined every second day were normal. Four samples each of blood, urine and stool collected between April 14 and May 9 did not reveal shigellae or other pathogenic organisms.

Blood agglutination tests for shigellae carried out with the aid of the routine technic and the method of Gonzales and Morales Otero were negative on April 15. *Sh. alcalescens* agglutinins were found, however, on May 9. The serum of the patient agglutinated *Sh. alcalescens* stock strains in a dilution 1:40 and 1:80 and the *Sh. alcalescens* strain isolated from the patient in a dilution of 1:320. The organism isolated from the patient's blood and urine was morphologically, biochemically and serologically identical with *Shigella alcalescens* subtype "A" of Neter.⁸ It was inhibited by 2 mg. per 100 ml. sulfadiazine *in vitro*. As previously mentioned, agglutinins developed in the patient's blood several weeks after infection, in spite of the early and successful treatment with sulfadiazine.

SUMMARY

Shigella alcalescens was cultured from the blood and urine of a young woman with symptoms of pyelocystitis, diarrhea, chill and fever. The organism was markedly sensitive to sulfadiazine and the infection was completely and rapidly cured by this drug. Agglutinins to the organisms developed several weeks later.

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INTESTINAL COCCIDIOSIS

REPORT OF TWO CASES OF *ISOSPORA HOMINIS**

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In October 1944, two men in the same company of a medical detachment stationed at Espiritu Santo, New Hebrides, were found, on routine examination of stools, to be harboring *Isoospora hominis*. Their division had been in the Saipan Campaign.

Rivolta,¹ in 1878, first described the sporozoan, *Isoospora hominis*, found in the stools of man. In 1935, Magath,² at the Mayo Clinic, collected from the literature only 200 authentic cases with intestinal infection by this sporozoan. Craig,³ in his book on *Protozoan Diseases*, stated that he had never seen an instance of this type of intestinal infection. Albritton and Fitzwater⁴ reported an infection in a soldier in New Guinea who received two treatments with tetrachlorethylene, following which the organisms disappeared from the stools. Kiskaddon and Renshaw⁵ found reports of an additional 25 cases and added one case of coccidiosis in a 60 year old man which was associated with an ulcerative colitis. The infection cleared spontaneously with no specific treatment other than that for the ulcerative colitis.

Although most infections of *Isoospora hominis* are accidentally encountered during routine stool examination, without any associated clinical symptoms, there is a small group of cases in which the organism appears to be pathogenic for man and causes intestinal symptoms. The two infections described here were encountered during 5000 routine examinations of stools for parasites and are being reported because of the scarcity of this type of infection and because of the questionable pathogenicity of the organism.

REPORT OF CASES

Case 1

A soldier, age 21 years, who served during the Saipan Campaign in a medical battalion during June, July and August of 1944, was admitted to a station hospital at Espiritu Santo on September 18 because of a cough and fever. While there, he developed diarrhea which subsided after five days under morphine therapy, but which recurred on October 1. He stated that for the previous six months he had noticed a cough, fever and malaise and had lost twelve pounds in weight. He was transferred to a general hospital on October 9, still complaining of cough, fever and intermittent diarrhea. Physical examination was essentially negative and the patient did not appear acutely ill.

The blood leukocyte count was 11,850, erythrocyte count, 4,460,000, and hemoglobin 90 per cent. The differential count showed 74 per cent polymorphonuclears, 17 per cent lymphocytes and 9 per cent eosinophils. The sedimentation rate was 10 mm. for one hour and the volume of packed cells was 50 mm. per 100 ml. of blood. The urinalysis was essentially normal. Stool examination was negative for hookworm and, on October 17, was

* Received for publication, October 8, 1947.

positive for *Isospora hominis*. Both oocysts and sporocysts were found by the flotation method. The stools remained positive until October 23. Repeated sputum examinations were negative for fungi and tubercle bacilli. On October 16, the patient was given sulfaguanidine. The diarrhea stopped and the final stool examination, on November 4, became negative for parasites and ova.

Case 2

A 24 year old soldier, a member of a medical battalion, was admitted to the hospital, September 28, 1944. While on Saipan, in July 1944, he developed a diarrhea for nine days, having about five bowel movements a day associated with abdominal cramps. In August, he had a recurrence of diarrhea for three days and was given bismuth, paregoric and sulfaguanidine. He was admitted to a psychiatric ward because of headaches and nervousness. There was no associated diarrhea at this time. The physical examination disclosed no un-

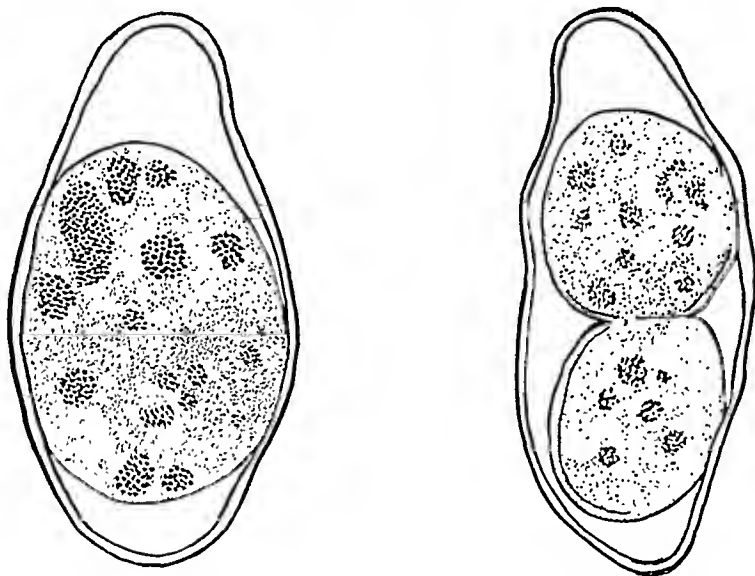


FIG. 1. Stage I (left), drawing of an oocyst of *Isospora hominis* in fresh stool with its large central nuclear mass of cytoplasm enclosed in an outer refractile double wall. Stage II (right), beginning division of the cyst into two sporoblasts.

usual findings. On September 28, stool examination revealed both oocysts and sporocysts of *Isospora hominis*. The stool remained positive until October 7. He was given bismuth salicylate and saline enemas daily for five days. The stools became negative immediately thereafter. The blood showed no abnormal findings except for an increased eosinophil count of 6 per cent. The urine was essentially negative.

DISCUSSION

In view of the fact that both soldiers had been in the same medical detachment, the stools of 51 soldiers from the same outfit were examined for similar parasites, but were found to be negative. Both cases illustrate the pathogenicity of this type of intestinal infestation in man and its ability to produce clinical symptoms. Eosinophilia was present in both patients and treatment promptly caused the disappearance of the cysts from the stools. It is important to differentiate the cysts of *Isospora hominis* from the eggs of helminths.

Isospora hominis, which is found not only in man, but also in the cat and dog, grows within the epithelial cells of the intestines and multiplies asexually. Fertilization occurs within the lumen of the bowel, and it is the female macrogamete that develops into the oocyte (Fig. 1, Stage I) and is passed into the feces. This is known as the infective stage.

The cysts of *Isospora hominis* are ovoid, elliptical, refractile bodies usually measuring about $28 \times 14 \mu$. They have a clear wall with two layers. The ends are usually rounded but occasionally one end is constricted and has a knoblike protrusion. The nucleus occupies most of the cyst and contains much granular material and several clear vacuoles. The contents of the cyst divide into two or four secondary cysts (Stage II) or sporocytes. In our specimens only cysts with two spores were seen. We preserved stool specimens in merthiolate for as long as sixty-six days at room temperature and both stages were found during the entire period. The stool specimens were finally discarded because of an overgrowth by yeast and mold.

Various types of therapy have been used for removal of the cysts from the feces. The following methods of treatment have been used with satisfactory results: (1) large doses of bismuth salicylate plus enemas of 2 per cent sodium bicarbonate; (2) bismuth salicylate and charcoal three times daily; (3) sulfaguanidine and (4) tetrachlorethylene. It is well known, however, that infection with *Isospora hominis* may be self-limited.

SUMMARY

During the course of examining approximately 5000 stool specimens for parasites, in a general hospital at Espiritu Santo in the New Hebrides, two infections with *Isospora hominis* were encountered. Both soldiers were members of the same company, both manifested mild intestinal symptoms of intermittent diarrhea, malaise and loss of weight, and both responded promptly to treatment. Eosinophilia was present in both patients. The oocysts and sporocysts were the only stages seen in both the fresh stool specimens and in specimens kept at room temperature for a period of sixty-six days. The cysts were easily identified in the fresh feces emulsified in water and also by the flotation method, using zinc sulfate or table salt.

Acknowledgment. The technical work was performed by Raymond R. Crandell, technician fourth grade.

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CLINICOPATHOLOGIC CONFERENCE*

E. T. BELL, M.D.

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CLINICAL DATA

The patient was a 57 year old white woman who was admitted to the hospital on October 16, 1942 and expired on March 14, 1943.

History. The patient was first seen in this hospital on March 19, 1940, and was discharged nearly one year later in January 1941. At that time she complained of an ulcer on the left leg of fifteen months' duration. Biopsy of the ulcer showed a chronic suppurative inflammatory reaction. The ulcer did not respond to conservative therapy, so the patient was transferred to the Surgical Service and amputation of the left leg was done 4 cm. above the condyle of the femur. It was thought that the ulcer was probably due to an old deep thrombophlebitis. The patient subsequently developed pain in the left flank. A diagnosis of renal calculus in the upper calyx of the left kidney was made and, on December 12, 1940, a left nephrolithotomy was performed with an upper left calyceal resection. The patient was then transferred to a convalescent home.

She was again admitted to the hospital on April 28, 1941, on the Dermatology Service because of a purulent drainage from a subcutaneous nodule of the right thigh. She was discharged after seven days and was subsequently followed in the Out-Patient Department because of ulcerative lesions on the left thigh and because of the appearance of similar lesions on the right thigh in June 1942.

At the time of her last admission, on October 16, 1942, the patient complained of ulcerative lesions on the right leg of two to three months' duration. She stated that fifteen or seventeen ulcerative lesions had formed on the right lower extremity during the previous six months, and seven or eight on the left thigh during the same period. She complained of burning pain in the area of the ulcers for the previous three or four weeks. The past history revealed that the patient had been struck by lightning when she was 17 years old and had suffered burns of both legs and the right arm; that she had had a phlebitis at the age of 25; and a cholecystectomy because of a cholelithiasis at the age of 42. The history was otherwise noncontributory.

Physical examination revealed a well developed and well nourished white woman with a blood pressure of 132/78, temperature 98.8 F., pulse rate 80 and respiratory rate 18 per minute. Examination of the head and neck was negative. The lungs were clear and the heart was negative to auscultation and percussion. Examination of the abdomen revealed the liver to be palpable one fingerbreadth below the costal margin. Examination of the extremities revealed the left leg to be amputated just above the condyle of the femur. There were many pig-

* Received for publication, October 18, 1947.

mented areas over the calf of the right leg and one on the left thigh. There was a punched-out ulcer on the medial aspect of the stump of the left leg with an indurated inflamed area surrounding it. On the right thigh anteriorly there was a large erythematous plaque covering most of the anterior surface. On this plaque there were four punched-out ulcers with serpiginous outlines. The edges of the ulcers were undermined.

Laboratory findings. The blood serology was negative. The hemoglobin was 86 per cent, the leukocyte count 6800 with 66 per cent neutrophils, 17 per cent lymphocytes, 8 per cent monocytes, 8 per cent eosinophils and 1 per cent basophils. Morphology of the blood cells was normal. Throughout the hospital admission, the urinalyses showed a few pus cells but were otherwise negative. An x-ray film of the chest taken on October 20, 1942, was normal.

Hospital course. She was given a course of treatment with superficial x-ray to the ulcerative lesions which responded slowly to treatment. The patient subsequently developed lesions on the back which were also treated with x-ray. About two months after admission the ulcerative areas seemed to be fairly well healed but thereafter ulceration occasionally recurred. In the first part of 1943, the patient developed fever and complained of pain in the right side of the chest with associated cough. At that time there were decreased breath sounds and dullness to percussion in the right lower portion of the chest. X-ray films showed fluid in the base of the right side of the chest cavity with congestion and consolidation in that area. A diagnosis of pneumonia of the right lower lobe was made.

Typing of the sputum was done and a type XVII Pneumococcus was found. The patient was given a course of sulfathiazole and, three days later, showed marked improvement and return of the temperature to normal. The patient then developed a fever up to 100 F., with associated persistent cough but with no chest pain. There was a friction rub over the right posterior and lateral portions of the chest with diminished breath sounds at the right base. X-ray films at this time showed an area of density in the right cardiophrenic angle and a rounded mass in the posterior part of the chest. Examination of the sputum showed no acid-fast bacilli. On February 24, 1943, a thoracentesis yielded fluid which showed a count of 2365 white cells with 21 per cent neutrophils and 79 per cent mononuclears. Culture of the fluid yielded no growth. Subsequent thoracenteses showed essentially the same findings and all examinations were negative for acid-fast bacilli. On March 11, the patient suddenly developed a temperature of 103 F., and her condition steadily became worse. She began to raise a blood-tinged sputum and complained of severe pain on the right side. Her condition rapidly became critical and she expired on the one hundred sixty-eighth hospital day.

Treatment for the most part consisted of x-ray irradiation to the ulcers of the legs. Because of the chronic ulcers of the leg she also received a course of treatment with mercury succinimide. The remainder of the therapy was largely supportive.

Clinical diagnosis. The final clinical diagnosis was mycosis fungoides but this diagnosis was not made until multiple lesions had appeared.

POSTMORTEM FINDINGS

Autopsy findings. At autopsy 1000 cc. of blood-stained fluid was present in the right pleural cavity. The right lung weighed 1120 Gm. and contained a tumor 8 cm. in diameter in the lower lobe, as well as several small abscesses. There were no other important gross lesions in the viscera.

Microscopic sections of the lung tumor had the same structure as the lesions of the skin, viz., the appearance of a reticulosarcoma. Microscopic nodules of the same structure were found in the kidneys and the liver.

COMMENT

Mycosis fungoides is a malignant lymphoblastoma confined largely to the skin, but at autopsy visceral lesions are usually found. The diagnosis is made on the basis of the clinical features and on biopsy of tissues which show a malignant lymphoblastoma resembling reticulosarcoma. The original single ulcer of the left leg was not recognized as mycosis fungoides by the pathologist because of the associated suppurative inflammation. The amputation was, of course, unnecessary.

EDITORIALS

THE ROLE OF THE PATHOLOGIST IN WORLD HEALTH*

There is a real and growing appreciation of the essential part played by the pathologist in the development of modern scientific medical technics and I can confidently predict an even more essential role, and a correspondingly increased responsibility, for the pathologist in the future shaping of scientific medicine. I should like to suggest some of the responsibilities and some of the possibilities which the pathologist will have in world medicine and therefore, of course, in the promotion of world health.

The clinical pathologist should take the lead in the establishment of an international nongovernmental body speaking for pathologists, which would work with the World Health Organization (WHO). The Interim Commission of this Organization (WHO.IC) now works with governmental health administrations and, eventually, will have official relationships with such international nongovernmental organizations.

I should like to point out to you that there is no pathologist, as yet, on the international Expert Committee for the Preparation of the Sixth Decennial Revision of the International Lists of Diseases and Causes of Death. This deficiency should be remedied.

There must be a liaison between the legally constituted specialized agency of the United Nations which deals with international health matters, namely the World Health Organization (WHO), and your national organization of pathologists through some world federation of clinical pathologists, such as was recently envisaged by the British proposal to expand the European Association of Clinical Pathologists to world-wide membership. I submit that your Society should take the lead in effecting such a federation.

What exactly would a "World Federation of Clinical Pathologists" do? The following are only a few possibilities and I am sure that you will be able to elaborate on them. Let me begin with one of the simplest and most necessary tasks.

1. *Nomenclature.* A pathologist should, in my opinion, be a member of the Committee for the Revision of the International Lists. This is most important. I shall but mention the chaos in bacteriologic nomenclature, the well-known plurality of terminology in simple blood grouping, and the confused position with respect to the terminology of the Rh factor.

A higher standard throughout the world in ascertaining the causes of disease and death is needed. The statisticians are doing a very good job, but how good can their work really be without proper education of those, such as the pathologists, who provide the original "raw material" for their work? Let me mention a few other functions of this hypothetical "World Federation of Clinical Pathologists".

* Excerpt of an address delivered at the Twenty-Sixth Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 28, 1947.

2. *Education.* WHO already has financed 200 annual fellowships from many countries to other countries. We need many more such fellowships embracing a greater group of countries. The proposed Federation could make a great contribution to the rapid world-wide spread of the newest scientific ideas. The desirability of extending the use of exact methods and postmortem technics in investigating the end-results of disease is obvious. Surely the time has come to develop the idea that medical progress is connected with the human quality of doctors being liable to err, and to uncover any ignorance such as may be cloaked by obscure terminology. Surely the time has come for the proper recognition of the true worth of scientific workers, among whom are so many pathologists. This educational approach is probably the only method by which a large and useful influx of workers may be expected to perpetuate the high research ideals of Pathology.

3. *Legal medicine.* It is time that pathologists speak out in world circles on the definition of a medicolegal expert. In most instances the medicolegal expert should be a pathologist. Pathologists should also have their views considered on such subjects as the end-results of medical programs, employees' compensation and other similar questions which are now the subject of intensive debate in national and international circles.

4. *Research.* This field is so enormous that I shall mention only a few topics which are even now, in the interim stage of WHO, under active survey, and I am sure that you will agree that there is in each of these a very definite part for you to play: influenza, cancer, nutrition, kala-azar, arteriosclerosis and heart disease.

The opportunity to participate and to take leadership in world scientific matters, therefore, lies open to the American Society of Clinical Pathologists.

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PROPER USAGE OF THE TERM "LEUKEMIA"

Any individual concerned with the study of hematologic disorders will recall innumerable instances of the improper application of the term "leukemia". Although hematologists and pathologists are familiar with the frequent disparity between clinical phenomena and underlying pathologic lesions, many physicians are prone to utilize inadequate criteria in the diagnosis of blood dyscrasias. The appearance of abnormal leukocytes in the circulating blood, alone, or in combination with alterations in total white and/or red cell content, lymph node enlargement, splenomegaly and hepatomegaly are not absolute indications of the presence of leukemia.

Modern texts define leukemia as a lethal disease of the blood-forming organs characterized by marrow and visceral infiltration by tumor-like overgrowths of one or another cellular component of the hematopoietic system. There are usually, but not necessarily, abnormal leukocytes in varying numbers in the

peripheral blood. Such a concept indicates a primary disease of the hemato-poietic system, notably the bone marrow, and accords to the circulating blood a wholly dependent and subordinate status. Although frequently an index of bone marrow activity, the blood stream is an unreliable source for evidence of the nature of hematopoietic dysfunction.

It is common knowledge that the pancytopenia of pernicious anemia reflects not a sparsely populated marrow as was earlier presumed, but rather an intense hyperplasia of red blood cell precursors which fail to mature or gain egress from the medullary cavity. An analogous situation for white blood cells prevails in malignant neutropenia wherein there is interruption of maturation of neutrophils with medullary myeloid hyperplasia although peripheral blood contains few or no granulocytes. Even "aplastic anemia" which was originally conceived to develop on the basis of depletion or agenesis of all marrow elements, has been found with critical study actually to represent a congeries of diseases in which marrow content varies from an acellular state to one of marked hypercellularity.

Thus attention to the tissue changes at the root of dyscrasias manifesting diminution of cellular elements in the blood has demonstrated the lack of uniform correlation between blood smear and bone marrow. In parallel fashion, the presence of excessive numbers of normal and abnormal leukocytes in the circulation has also frequently been found to represent incorrectly the actual state of hematopoiesis.

In disease states the marrow and other components of the blood-forming organ contribute to the blood picture in a less predictable fashion than was hitherto supposed. As a result there have been evolved many syndromes; among them, the leukemoid states, nonleukemic myelosis, myelophthisic anemia and leuko-sarcoma, not to mention the leukemic, subleukemic, aleukemic and nonleukemic phases of leukemia. In all of these conditions variable blood pictures appear and they are, even in the hands of well trained hematologists, confused with one another.

For a number of years it has been shown that the marrow content of the sternum is accessible through the medium of the biopsy trephine and even more readily, the aspiration needle. Countless studies have attested to the facility, safety and value of sternal marrow aspiration. Since the peripheral blood represents an unreliable factor in the establishment of definitive hematologic diagnosis, and the bone marrow so often indicates the fundamental lesion of leukemia, certainly it would appear to represent the likely site for conclusive diagnostic investigation. The diagnosis "leukemia", based as it is so frequently upon misleading observations, should be made, it is felt, only in those cases in which bone marrow study has been completed, and this, regardless of the character of the clinical picture.

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THE PRACTICE OF PATHOLOGY IN THE TUMOR CLINIC

The demand for early recognition of cancer places a responsibility to the patient and his physician on the clinical pathologist. Cancer is a disease which

is readily identified when an entire lesion or organ is available for study; however, interpretation of findings on biopsy alone is often more difficult and fails to establish the true nature of the disease in a certain percentage of cases. Failure results most commonly because the pathologist receives an inadequate specimen or incomplete clinical information. These deficiencies may be avoided in tumor clinic practice if the pathologist, himself, removes the specimen for biopsy. Because the majority of samples can be taken in the laboratory and a minimum of time and equipment is required, there is little hardship and many advantages for the pathologist who assumes this task. He is provided the opportunity of studying patients, and information concerning clinical features of a disease may be elicited which is necessary for the correct interpretation of microscopic changes. A knowledge of the natural history of cancer, as well as an understanding of methods of cancer therapy are acquired from clinical association with the patient. The pathologist is also more capable of selecting representative tissue from a lesion and he appreciates the necessity of removing it without trauma and of insuring proper and prompt fixation.

Removal of the tissue need not be a complicated operation, since small specimens are often adequate when they have been properly selected and carefully handled. Local anesthesia is frequently unnecessary and sutures should not be placed through neoplastic tissue. Light desiccation adequately seals the surfaces and prevents the dissemination of cancer cells. Most specimens of lesions on the skin, as well as on the lips, tongue, buccal mucosa, vulva and cervix can be excised with the scalpel and lifted on the knife blade to avoid trauma from forceps. Samples from the posterior portion of the tongue, pharynx, nasal cavity and rectum are removed with sharp biting forceps of a type designed to minimize compression. Since the site for removal of tissue is always directly visualized, oozing is controlled by the use of pressure, desiccation or Monsel's solution.

Needle "biopsies" are particularly useful when it is desirable to avoid incision. In tumor clinic practice, this method has been successful in establishing the diagnosis in a high percentage of patients with malignancy of breast, subcutaneous tissue or lymph nodes before surgical treatment or radiotherapy is planned. Pieces of tissue that are satisfactory for paraffin sectioning can be aspirated from soft tumors through an 18-gauge needle, and in firm tissue, the Silverman biopsy needle is used to extract a solid core of material. It is desirable for the pathologist to remove these specimens in the laboratory because he can quickly ascertain whether satisfactory tissue has been obtained and repeat the puncture if necessary.

The examination of secretions and excretions for the presence of cancer cells has given very useful and specific information in certain types of lesions. One may perfect these techniques by applying them to the related clinical problems. The practice of pathology as it has been described in the tumor clinic enables the pathologist to carry his share of the responsibility and places him in a more favorable position to render service to the patient and the medical staff.

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SELECTED ABSTRACTS

Morphologic Changes in the Lymphocytes of Persons Exposed to Ionizing Radiation. ANNA-MAE DICKIE AND LOUIS H. HEMPELMANN. J. Lab. and Clin. Med., 32: 1045-1059, 1947.

The study was done on persons employed at the Los Alamos Scientific Laboratory and covered a two year period. Except for the control group, all were employed so that exposure to ionizing radiation or toxic chemicals resulted. Supravital staining was used and it was found that lymphocytes of the exposed persons contained more refractive neutral red bodies than did the cells of the controls. This is a quantitative and not a qualitative change, since these same bodies have been described in normal cells but in significantly smaller numbers. Similar increases in abnormal lymphocytes have been seen in rabbits and cows exposed to large doses of ionizing radiation.

There is a significant statistical decrease in the total leukocyte counts in the exposed group but the per cent of lymphocytes and the absolute number of them show no significant change.

This is one of probably numerous reports which will be appearing on the changes due to radiation. The correlation with other findings and their significance will have to wait on further investigations.

Dallas, Texas

J. H. BLACK

The Role of Phagocytosis in Resistance, as Related to Age of Granulocytes, Following Primary and Reinfection Studies with Hemolytic Streptococci in Macacus rhesus. SAMUEL SASLAW AND CHARLES A. DOAN. J. Lab. and Clin. Med., 32: 878-885, 1947.

Using a modified Huddleson's method a study was made in monkeys to determine variations in phagocytic properties of the circulating granulocytes of infected animals. Young cultures of *Streptococcus hemolyticus*, Group C, not resistant to phagocytosis and adjusted to 30 billion per ml. were used. Equal quantities of this emulsion were mixed in agglutination tubes with fresh blood and incubated at 37 C. for two hours. Thicksmears were made, fixed and stained and the phagocytic power measured by the number of organisms engulfed by 25 cells. The age of each cell, in terms of lobation of nucleus, was noted.

Earlier studies had shown that monkeys inoculated intranasally with this same organism reacted with marked peripheral leukocytosis but without elevation in the opsonic index. Reinoculation three to six months later showed a significant increase in phagocytosis without appreciable leukocytosis. In the present study these findings were corroborated and it is believed that resistance to primary infection consists chiefly of an increase in the number of available leukocytes, while in a second infection, resistance may occur without increase in the number of cells but with marked increase in their ability to engulf organisms. It was found also that the younger mature cells were definitely more effective as phagocytes than the older cells and that phagocytosis was related directly to the age and motility of the cells. The belief is expressed that there is a humoral catalyst which results from the primary inoculation and which facilitates phagocytosis so that fewer cells are needed to combat reinfection than are required for protection against the primary inoculation.

Dallas, Texas

HARVEY BLACK

Meningitis Leptospirosa. E. M. BUZZARD AND J. A. H. WYLIE. Lancet, 2: 417-420, 1947.

From the Radcliffe Infirmary, Oxford, 5 cases of meningitis are reported in which the blood serum gave positive agglutination reactions for *L. icterohemorrhagiae*. The titer rose from 1:10 in a week to 1:1000-2000 after a fortnight. All patients were young men who had either bathed in rivers or worked where there were many rats. Sudden onset, fever, vomiting, photophobia and marked conjunctival suffusion were prominent; the last two symptoms were especially striking. The sensorium was always clear and icterus was absent. Most of the commonly employed laboratory tests were negative. The cerebrospinal fluid was colorless and under slightly increased pressure and the white cells numbered from 50 to 3000; at first neutrophils predominated, but later lymphocytes were more numerous. The pro-

tein, glucose and chlorides of the fluid were unchanged and ordinary cultures remained sterile. There were no fatalities in the series. Experimental results tend to support the view that, in bathing, the leptospirae enter the blood stream through conjunctiva, nasal mucosa and fauces.

Fort Wayne, Indiana

S. M. RABSON

This Wormy World. NORMAN R. STOLL. J. Parasitol., 33: 1-18, 1947.

This presidential address read at the annual meeting of The American Society of Parasitologists in Boston last December should be of interest to everyone, even to the reader who is only remotely interested in parasitology. The nature of its contents precludes a suitable abstract in the space available. An interesting half hour's reading is guaranteed by your reviewer.

Rochester, New York

W. S. THOMAS

BOOK REVIEWS

Atlas of Cardiovascular Diseases. By IRVING J. TREIGER, M.D., Assistant Professor of Medicine, University of Illinois, Chicago; Cardiographie Department, Presbyterian Hospital, Chicago; Consulting Cardiologist, Municipal Tuberculosis Sanitarium, Chicago. 180 pp., 69 plates containing 244 illus., 11 in color. \$10.00. St. Louis: The C. V. Mosby Company, 1947.

This book is an attempt to correlate important information obtained from the history and physical examination with roentgenographie, electrocardiographic and pathologic findings in normal subjects and in a wide variety of cardiac and vascular abnormalities. The author, in the preface, very properly emphasizes the importance of the history and physical examination, as well as laboratory procedures, in the diagnosis and care of patients. The work is, in general, well arranged and the illustrations, particularly those of pathologic specimens, are excellent.

The reviewer is in some doubt as to what group of individuals might profit most by this atlas. It contains little with which an experienced clinician is not familiar and it is not sufficiently detailed to be of much value as a reference book. Medical students or those beginning their training in medicine after graduation may find the book useful, but the author has tried to cover a very broad field and much of the electrocardiographic treatment is inadequate and might be misleading. Many structural defects in the heart are often not associated with characteristic alterations in the electrocardiogram and this adds to the difficulties faced by an author of a book of this character. Students may easily obtain an incorrect idea of the value of such a tracing in the diagnosis of heart disease from an atlas type of presentation.

Many of the electrocardiograms presented in this book are poor examples of the condition they are supposed to illustrate and a number of them are faulty from the technical standpoint. Multiple precordial leads, rather than a single chest lead, would have added worthwhile information, particularly in several of the cases dealing with myocardial infarction. The terms, right and left ventricular strain, are employed in describing several electrocardiograms. Although these appear to be sanctioned in many quarters and are widely used, the writer does not believe they are justified since they imply the presence of a mechanical situation that cannot be estimated from an electrical record with any certainty.

In spite of the above criticisms the book does have many good features and many of the points raised might apply as well to any atlas, as to this particular volume.

Ann Arbor, Michigan

F. D. JOHNSTON

Penicillin Therapy Including Streptomycin, Tyrothricin and Other Antibiotic Therapy. Ed. 2. By JOHN A. KOLMER, M.D., DR.P.H., Sc.D., LL.D., L.H.D., Professor of Medicine, School of Medicine and School of Dentistry, Temple University; Director of the Research Institute of Cutaneous Medicine. 339 pp., 27 figs., 37 tables. \$6.00. New York: D. Appleton-Century Company, 1947.

In this second edition, Dr. Kolmer has included much new information about penicillin and the various antibiotics by extensively rewriting and reorganizing his popular monograph to include the latest theories on the action, pharmacology, toxicity, and principles of therapy.

Those aspects of the pharmacology of penicillin having to do with its absorption, diffusion and excretion and its toxicity, have been brought into line with latest developments. Present concepts regarding the exact mechanism of antimicrobial activity are clearly presented, and in his discussion of this subject, which has commanded considerable recent attention, the author has given his own opinion concerning the proposed theories. He has reviewed recent reports dealing with levels of penicillin in different body fluids resulting from administration by various routes, including inhalation of aerosols, oral and parenteral, and use of peanut oil and beeswax mixtures.

The still open question of the advantages and disadvantages of penicillin and the sulfonamide compounds is briefly summarized, and attention is called to the paucity of reports bearing upon the comparative therapeutic properties of these substances in the treatment of infections in human beings due to pathogenic bacteria susceptible to these agents.

This volume will serve as a useful reference to those interested in methods for detecting and assaying various antibiotics, for it includes discussions concerning penicillinase, cystine hydrochloride, taka diastase, clarase and other inhibitors. The author has also included a discussion of the following antibiotic substances: bacitracin, penicillic acid, clavacin, notatin, penicidin, actinomycin, aspergillie acid, citrinin, fungicidin, flavacin, subtilin and chlorophyll. There is an unusually complete bibliography following each chapter. The clinician will find this volume a ready source for the current opinions regarding the use of streptomycin and the other antibiotics in the treatment of different diseases by these agents.

Eloise, Michigan

CHARLEY J. SMYTH

Diseases of the Chest with Emphasis on X-Ray Diagnosis. By ELI H. RUBIN, M.D., Attending Physician, Division of Pulmonary Diseases, Montefiore Hospital and Country Sanatorium, New York; Visiting Physician in Tuberculosis and Physician-in-Charge, Chest Clinic, Morrisania City Hospital, New York. *The Principles of Surgical Treatment.* By MORRIS RUBIN, M.D., Assistant Visiting Surgeon, Triboro Hospital and Morrisania City Hospital, New York. 685 pp., 355 figs., 24 in color. \$12.00. Philadelphia and London: W. B. Saunders Company, 1947.

This book should be a welcome addition to any library on chest medicine. It has the possibility of being a successor to Fishberg's *Pulmonary Tuberculosis* of the last generation. Although it is vastly different from that eminent work, the differences between the two books virtually express the changing conditions as well as the march of progress in the study of chest diseases over the last half century. The authors state that the book is "intended for use by general practitioners, sanatorium physicians, medical students and radiologists, emphasis being placed on X-ray diagnosis".

There are six main sections, each of which is divided into four to nine chapters, with a total of 37 chapters. The first section deals with principles; the second, with acute and chronic pneumonias and pneumonitis; the third, with tuberculosis; the fourth, with bronchial diseases; the fifth, with other contiguous structures, including mediastinum, diaphragm and pleura; and the sixth with surgical principles, surgical technic being only briefly discussed.

The discussions are presented in logical order of relation of cause to effect and are followed in turn by treatment. They are clear, concise and generally correct. They rarely diverge from a charted course or elaborate too much on controversy. The various aspects of obscure problems are mentioned without conclusions. The reader is allowed to form his own opinions. It is noteworthy that there are so few places where additional information could be added in the space allowed. Even most recent procedures are brought up to the moment of publication.

The chapter on emphysema, asthma, pulmonary cysts and heart-lung diseases are outstanding and timely because these conditions have caused, and still cause, much confusion in the diagnosis and treatment of diseases of the chest. The chapter on surgical principles is what it is intended to be, a fine supplement to a medical treatise.

To add any constructive suggestions, one is obliged to resort to minor details. Some suggestions and points of historical interest which could be mentioned for diseases in this geographic region are: plague pneumonia, measles bronchopneumonia; the presence of rice water or gruelly sputum in monilia; a more complete discussion on the disease histoplasmosis; Simon's apical hematogenous foci, Assmann's subapical foci; the adrenal syndrome in chronic tuberculosis; hepatic damage other than amyloidosis in pulmonary tuberculosis;

pulmonary adenomatosis; hamartomas of the bronchi; and other reticulo-endothelial malignant diseases besides Hodgkin's disease.

The mechanism of formation and location of early tuberculous cavities could be elaborated on to advantage. It should also be pointed out that in addition to direct extension, most tuberculous pericarditis in young persons or in aboriginal peoples is probably due to retrograde lymph flow from the anterior (caval) and posterior (Botalli) lymph nodes rather than by direct extension.

It might be further suggested that the subject of pneumoconiosis with silicosis as the dominant condition might be extended to show the effect of mixed processes. Owing to the great number of interfering dusts and infections, there is not a constant disease pattern but usually an unpredictable disease complex with regard to size, location, quality and quantity.

The pathologic discussions are of necessity limited because the work is clinical. Nevertheless, there are places where a little more pathologic discussion would have added to the value of the text. Perhaps the greatest fault of all, in fact the only major fault, is the mediocre to poor type of reproductions of the gross pathologic specimens.

There should be a special commendation, however, for the way the Doctors Rubin have used the famous Ciba color plates by Dr. Frank Netter. Instead of passing these masterpieces by as just advertisements of a drug house, they have preserved them for posterity in a classic textbook. The fact that some of the illustrations are partly schematic does not detract from their value. The roentgenograms are also representative of good quality and are quite adequate to meet the needs of the book.

The bibliographies at the end of each chapter are well chosen and are arranged in proper groupings and in alphabetical order. With great discernment, the authors have usually selected from the massive literature, one or a few of the important references. There is a table of contents and a good index.

The publisher's art is of the best, with generally a good readable print arranged in two columns on the finest glazed book stock paper. The binding is up to standard.

In conclusion, it should be said that the aims of the authors have been achieved and more, the book should be useful to all chest specialists, even chest surgeons, as well as the other groups mentioned.

Chicago

HENRY C. SWEANY

Gynecology Including Female Urology. Ed. 2. By LAWRENCE R. WHARTON, PH.D., M.D., Assistant Professor of Gynecology, The Johns Hopkins Medical School; Assistant Attending Gynecologist, The Johns Hopkins Hospital; Consultant in Gynecology, The Union Memorial Hospital, Hospital for the Women of Maryland, Sinai Hospital and Church Home and Infirmary. 1027 pp., 479 figs., \$10.00. Philadelphia and London: W. B. Saunders Company, 1947.

This new edition of a text that first appeared in 1943 has been expanded to include the chemotherapeutic and antibiotic handling of the problems in gynecology and urology. The sections on Embryology and Congenital Malformations have been almost completely rewritten.

As in many textbooks, an attempt has been made to approach the subject too broadly. Section I deals with the anatomy of the female pelvis and the abdominal wall. Section II covers Embryology and Congenital Malformations. It is impossible to condense two such important subjects into 70 pages; consequently the presentation is too brief for the advanced student, but does give essential facts for the busy practitioner in a succinct manner.

The endocrinology of gynecology is much more completely covered and is correlated in a practical manner that is excellent. The author's real forte is evident in the well organized and most satisfactory sections on clinical and surgical gynecology. While the book is not intended to be a text on operative technique, an introduction is given to the common surgical procedures which is adequate for the student.

By including female urology, the author has emphasized that the problems of the repro-

ductive and urinary systems are frequently inseparable. A knowledge of female urology is indispensable to the gynecologist, just as a knowledge of gynecology is indispensable to the urologist in the treatment of female patients. Air cystoscopy with the Kelly cystoscope is an easily acquired technic and may be of inestimable value to the gynecologist in his evaluation of a patient's complaint. A chapter on water cystoscopy has been added, written by Charles L. Prince. It briefly outlines what every gynecologist should know about this procedure. Among the other chapters on urologic subjects, the chapter on "Pyelitis of Pregnancy" is outstanding. The Traut ambulatory treatment is outlined. The indications for ureteral catheterization may not be the same as advocated by other urologists but no one can disagree widely with the fundamental tenets outlined.

The illustrations are, in general, excellent. Many of them are familiar, having previously appeared in Kelly's *Operative Gynecology* and elsewhere.

The book possesses easy readability which in any scientific text is a desired asset. It will probably gain wide usage since it is one of our good elementary gynecologic texts and is authoritatively written.

Detroit

D. C. BEAVER

M. S. SHARP

Précis de Diagnostic Hématologique. By G. HEMMELER, Privat-docent à la Faculté de Médecine de l'Université de Lausanne, Chef de Clinique médicale. 168 pp. 15 color plates. 700 francs. Lausanne: F. Roth & Co., 1947.

The purpose of this synopsis of hematologic diagnosis is to "teach those fundamentals which permit an exact diagnosis by means of a blood smear". An introduction by Prof. Louis Michaud emphasizes the importance of morphologic hematology for medical students and practitioners. The book is divided into 6 chapters.

Chapter 1 (12 pages) deals with hematologic technic. This is, in the reviewer's opinion, the weakest part. The importance of hematocrit, photo-electric methods (at least for hemoglobin) are not mentioned, while three methods for peroxidase reaction and procedure for determination of blood iron are given. For enumeration of erythrocytes the reader is referred to instructions supplied by commercial houses. Chapter 2 (35 pages) is devoted to diseases affecting the erythropoiesis. Normochromic, hyperchromic, hypochromic and hemolytic anemias, erythroblastoses and erythrocytoses are discussed. Erythroblastosis fetalis, including the Rh factor, occupies only 24 lines. Chapter 3 (32 pages) deals with diseases affecting the leukopoiesis and contains a discussion of leukocytoses, leukopenias and leukemias. One misses a consideration of leukemoid reactions. Chapter 4 (7 pages) is devoted to diseases affecting the thrombopoiesis and Chapter 5 (3 pages) to hemorrhagic diatheses without thrombocytopenia. The final chapter 6 (19 pages) discusses treatment. Folic acid is briefly mentioned. Treatment with radioactive phosphorus, particularly of polycythemia vera is not mentioned, probably because it was not yet available at the European clinics.

A valuable feature for students is the brief summary following each disease, including clinical symptoms, blood picture, bone marrow findings and treatment. The value of the book is greatly enhanced by 15 plates containing 60 color photomicrographs of various hematologic conditions including concise descriptions (30 pages). These small photomicrographs are well selected and are of excellent quality, even by American standards. No bibliography is included.

This book should serve its purpose well for the French-speaking medical world. Some desirable changes and additions could easily be accomplished in a future edition.

Terre Haute, Indiana

LEON L. BLUM

State Central Case Record Systems and Local Case Registers for Tuberculosis. 88 pp., 18 figs., 4 tables. Washington: Federal Security Agency, U. S. Public Health Service, 1947.

Although neither of these two projects has been in operation long, it is advised that a central case record system is workable in states with a population of four million or less,

and that this system can be controlled in much the same manner as a local tuberculosis case register.

The usefulness of case registers for local health departments serving cities or counties has been amply demonstrated. Full time health officers, clinic facilities and adequate public health nursing staffs are necessary for its successful operation. A state control case record system for tuberculosis offers a practical way for accomplishing those purposes so vital to a state-wide tuberculosis program.

Factors that influence the size of the task and volume of work are: (1) prevalence of tuberculosis; (2) extent of case finding (mass x-ray service); (3) quality of reporting; (4) amount of service for tuberculosis, *i.e.*, sanatoriums, clinics, nursing and other field services, laboratories and rehabilitation; (5) size and type of population.

A register is recommended only if the local health workers actively support this installation. It provides a follow-up of suspects discovered by mass x-rays and a state-wide clearing center of information. It would give the state tuberculosis division a summary of the medical and supervisory status of cases in each subdivision of the state. It would also assist in the follow-up; give supervision of cases in those areas without local health departments; plan the state-wide tuberculosis control program; and provide assistance in the planning of the state budget. Cooperation of the local agencies is necessary for its success.

Eloise, Michigan

A. P. DERBY

The American Illustrated Medical Dictionary. Ed. 21. By W. A. NEWMAN DORLAND, M.A., M.D., Lieutenant Colonel, M.R.C., U. S. Army, Member of Committee on Nomenclature and Classification of Diseases of American Medical Association, Editor of "American Pocket Medical Dictionary". 1660 pp., 880 illus. \$8.00 without thumb index; \$8.50 with thumb index. Philadelphia and London: W. B. Saunders Company, 1947.

This edition contains many additions to medical terminology that have accumulated during the war years in the fields of war medicine and surgery, tropical medicine, aviation medicine, medical zoology and mycology, biochemistry and pharmacology, physics and nucleonics. Included among these are antibiotics, enzymes, vitamins and endocrines and medical applications of radioactive isotopes of the chemical elements. Many proprietary medicines, which were listed in previous editions and which have become obsolete, have been omitted. The key to pronunciation and the derivation of each word are given. There are 88 illustrations, including 233 portraits. This dictionary continues to be indispensable and most up-to-date.

NEWS AND NOTICES

THE TWENTY-SIXTH ANNUAL MEETING

The largest and by far the most successful meeting of the American Society of Clinical Pathologists was held in Chicago at The Drake Hotel, October 27, 28, 29 and 30, 1947. The total registration was 613, the attendance at the seminar was 313, at the banquet 356 and at the business meeting 192.

On Monday, October 27, demonstrations were held at different Chicago hospitals on Sternal Puncture, Rh Factor, the Electron Microscope and Photometric Analytic Methods. On Tuesday and Wednesday, October 28 and 29, a total of 29 papers were read, representing many new concepts, technics and evaluations. In addition, 12 papers were read by title. The business meeting was held on Tuesday evening and the banquet on Wednesday evening. At the banquet, the Society was honored in having as its guest, Dr. Edward L. Bortz, President of the American Medical Association. Dr. Bortz presented an address entitled "Medicine's New Frontiers". On Thursday, October 30, a seminar on "Diseases of the Kidney" was conducted by Dr. Balduin Lucké, of Philadelphia, and Dr. Arthur C. Allen of New York. Of the 547 who registered for the seminar, 313 were in attendance.

Election of Officers

The following Fellows of the American Society of Clinical Pathologists were elected to office:

President-elect, Dr. Osborne A. Brines, Detroit

Vice-president, Dr. Clyde G. Culbertson, Indianapolis

Members of Executive Committee (for a term of three years), Dr. Leonard W. Larson, Bismarck, N. D., and Dr. Stanley P. Reimann, Philadelphia

Members of Board of Censors (for a term of three years), Dr. L. W. Diggs, Cleveland, and Dr. F. C. Coleman, Des Moines, Ia.

Members of Board of Registry (for a term of three years), Dr. Lall G. Montgomery, Muncie, Ind., and Dr. Arthur H. Wells, Duluth, Minn.

Awards

The Ward Burdick Award was presented to Dr. Charles Sheard, Rochester, Minnesota, for his contribution to medicine in the field of Photometry and Spectrophotometry.

Awards to scientific exhibitors were given as follows:

Gold medal to Dr. Arthur H. Wells, Duluth, Minn., for exhibit on "Clinicopathologic Conference. Case Reviews".

Silver medal to Dr. Albert L. McQuown and Dr. Emma S. Moss, New Orleans, for exhibit on "The Etiologic Agents of Mycotic Infections. Laboratory Methods of Identification and Classification".

Bronze medal to Dr. Milton G. Bohrod, Rochester, N. Y., for exhibit on "Medical Photography in a General Hospital".

Appointments to Advisory Editorial Board

The following Fellows of the American Society of Clinical Pathologists were appointed as additional members to the Advisory Editorial Board for the year 1948:

Osborne A. Brines, M.D., Professor of Pathology, Wayne University College of Medicine, Detroit,

Edwin W. Schultz, M.D., Professor of Bacteriology and Experimental Pathology, Stanford University School of Medicine, San Francisco,

Lawrence Berman, M.D., Associate Professor of Pathology, Wayne University College of Medicine, Detroit,

George Gomori, M.D., Assistant Professor of Medicine, University of Chicago School of Medicine, Chicago,

Herman J. Linn, M.D., Pathologist, Veterans Administration, Dearborn, Michigan.

New Members Elected to Regular Membership

Abel, Harold A., New York, New York
Ackerman, Milton, Aspinwall, Pennsylvania
Adams, George, Bethesda, Maryland
Akerson, Irving B., Bridgeport, Connecticut
Allen, Pliny A., Omaha, Nebraska
Aronson, Roland S., Pittsburgh, California
Auerbach, Stewart, Nashville, Tennessee
Auger, Carlton, Quebec, Canada
Baird, Elwood Erwin, Denver, Colorado
Beeman, Joseph A., Boise, Idaho
Bennett, Granville A., Chicago, Illinois
Berman, Lawrence, Detroit, Michigan
Bornstein, Frederick P., Herrin, Illinois
Bornstein, Siegbert, Oteen, North Carolina
Bostick, Warren L., San Francisco, California
Bratley, Forrest G., Jackson, Mississippi
Breyfogle, Herbert S., Richmond, Virginia
Burns, Edward L., Toledo, Ohio
Butt, Edward M., Los Angeles, California
Churg, Jacob, Paterson, New Jersey
Clarke, James Yandes, St. Paul, Minnesota
Cohen, Hilliard, New York, New York
Cohen, Sidney, Council Bluffs, Iowa
Corrigan, Marion C., Chicago, Illinois
Cross, K. R., Des Moines, Iowa
Crumrine, Ralph M., Inglewood, California
Darling, John P., Mason City, Iowa
Donnelly, Joseph L., Ft. Thomas, Kentucky
Drapiewski, John F., Wilkes-Barre, Pennsylvania
Ducey, Edward F., Grand Rapids, Michigan
Dunn, Robert C., Baltimore, Maryland
Dutra, Frank R., Cincinnati, Ohio
Edmonds, Henry W., Seattle, Washington
Edwards, Jesse E., Rochester, Minnesota
Ernst, Kenneth F., San Francisco, California
Fanger, Herbert, Boston, Massachusetts
Ferguson, Ralph L., Vermillion, South Dakota
Fidler, Herbert K., Vancouver, B. C., Canada
Fite, Franklin, Philadelphia, Pennsylvania
Fitzgerald, Patrick J., New York, New York
Fox, Lester M., Brooklyn, New York
Friedman, Melvin, San Francisco, California
Gannon, John R., Cincinnati, Ohio
Gardner, Lawrence W., Detroit, Michigan
Gary, James L., Perryville, Md.
Gasparian, H. M., Cornwall, New York
Gewanter, Aaron P., Jamaica, L. I., New York
Gilmore, Hugh R., Jr., Ft. George Meade, Maryland
Ginzler, Arthur M., New York, New York
Goldenberg, Morris, Chicago, Illinois
Gotwald, David K., Nashville, Tennessee
Greene, Ralph C., Memphis, Tennessee
Greiner, Daniel J., Memphis, Tennessee
Gross, Stanley, Mt. Vernon, New York

Gunter, June U., Durham, North Carolina
Hamilton, Robert C., Pittsburgh, Pennsylvania
Harmos, Oscar, Cleveland, Ohio
Harsh, Robert C., Marquette, Michigan
Haukoil, Robert S., Milwaukee, Wisconsin
Heller, Elwyn L., Pittsburgh, Pennsylvania
Helwig, Elson B., Washington, D. C.
Henderson, Donald G., Canton, Ohio
Henry, James W., Chicago, Illinois
Holyoke, John B., Hanover, New Hampshire
Hooker, John W., Wilmington, Delaware
Jacobson, Moses A., Downey, Illinois
Kabler, Paul W., Minneapolis, Minnesota
Kaplan, Leo, Los Angeles, California
Kawasaki, Isaac A., Honolulu, T. H.
Kesten, Homer D., White Plains, New York
Ketchum, Clarence W., Valdosta, Georgia
Kiarsis, Victor, New Bedford, Massachusetts
Kimmelstiel, Paul, Charlotte, North Carolina
Klam, Najeeb, Monroe, Louisiana
Koenig, Albert S., Ft. Smith, Arkansas
Kogan, Vitali, New York, New York
Konzelmann, John B. H., Philadelphia, Pennsylvania
Koster, Eugene F., Cleveland, Ohio
Lacy, George R., Pittsburgh, Pennsylvania
Larkum, Newton W., Temple, Texas
Lide, Thomas N., Pinchurst, North Carolina
Lippincott, Stuart W., Seattle, Washington
Llewellyn, Maxwell B., Minneapolis, Minnesota
Loeffler, Ernst, Chicago, Illinois
Madsen, Martha, Detroit, Michigan
McKinley, Hugh A., Highland Park, Illinois
McMeans, J. W., Florence, South Carolina
Menten, Maud L., Pittsburgh, Pennsylvania
Minekler, Jeff, Portland, Oregon
Monaco, A. Ralph, Washington, D. C.
Moragues, Vincent, St. Louis, Missouri
Mostofi, Fathollah K., El Paso, Texas
Norris, Robert F., Philadelphia, Pennsylvania
Olken, Harry G., Boston, Massachusetts
Park, James H., Boston, Massachusetts
Peasley, E. D., Asheville, North Carolina
Permar, Howard H., Pittsburgh, Pennsylvania
Pizzolato, Philip, New Orleans, Louisiana
Poppiti, Robert J., Miami Beach, Florida
Proskauer, George G., Akron, Ohio
Randall, Charles C., Nashville, Tennessee
Rasmussen, Ruth F., South Bend, Indiana
Reece, John C., Morganton, North Carolina
Rieker, Walter, Jr., Seattle, Washington
Rosenthal, Maurice, Phoenix, Arizona
Rubenstein, Victor G., Los Angeles, California

- Russell, William O., Santa Barbara, California
 Russi, Simon, Richmond, Virginia
 Saxton, John A., Jr., St. Louis, Missouri
 Sayet, Maxwell M., Miami Beach, Florida
 Schaefer, Leroy W., Sioux City, Iowa
 Schafer, Etheldred L., Madison, Wisconsin
 Schmidt, Edward C. H., Kansas City, Missouri
 Schnap, Emil H., New York, New York
 Schneider, Louis A., New York, New York
 Shaver, John S., Oakland, California
 Silberberg, Martin, St. Louis, Missouri
 Silberberg, Ruth, St. Louis, Missouri
 Simard, Ernest E., Monterey, California
 Snavelly, John G., Stamford, Connecticut
 Solomon, Cyril, New York, New York
 Sommer, Melvin L., Beverly Hills, California
 Speer, Francis D., New York, New York
 Spyker, Mitchell A., Columbus, Ohio
 Steer, Arthur, Denver, Colorado
 Stevens, Dorothy L., Johnson City, New York
 Stock, Aaron H., Pittsburgh, Pennsylvania
 Strauss, Arnold F., Norfolk, Virginia
 Stryker, Walter A., Detroit, Michigan
 Szanto, Paul B., Chicago, Illinois
 Tarlow, Lillian S., Chicago, Illinois
 Taylor, James S., Kingston, New York
 Trumbull, Merlin L., Beverly, Massachusetts
 Valentine, Eleanor H., Philadelphia, Pennsylvania
 Wadsworth, Richard C., Bangor, Maine
 Walker, Gerald C., Utica, New York
 Wallace, James E., Erie, Pennsylvania
 Wallerstein, Harry, New York, New York
 Wartman, William B., Chicago, Illinois
 Waugh, Theodore R., Montreal, Quebec
 Weiss, Leo, Elmira, New York
 Weland, Regis E., Cedar Rapids, Iowa
 Williams, Robert J., Providence, Rhode Island
 Witebsky, Ernest, Buffalo, New York
 Wood, Harold, St. Louis, Missouri
 Wyatt, Tyree C., Syracuse, New York
 Zamecheck, Norman, Boston, Massachusetts
 Zundell, Joseph L., Rochester, Minnesota

New Members Elected to Honorary Membership

Sellek, Antonio, Vedao, Habana, Cuba

New Members Elected to Associate Membership

Hull, Thomas G., Chicago, Illinois
 Kaye, Sidney, Richmond, Virginia
 Shapiro, Shepard, New York, New York

New Members Elected to Corresponding Membership

Montoya, Alberto, Medellin, Colombia, S. A.
 Torres, Ernani T., Bahia, Brazil, S. A.

New Members Elected to Junior Membership

Adelson, Lester, Hartford, Connecticut
 Beckett, Ronald S., Hartford, Connecticut
 Blaustein, Ancel, Schenectady, New York
 Boley, James O., Kansas City, Kansas
 Claudon, Dann B., Milwaukee, Wisconsin
 Collins, William T., Oakland, California
 Dietz, Paul C., Evanston, Illinois
 Drake, William L., Jr., Milwaukee, Wisconsin
 Ellis, John M., Oakland, California
 Grondahl, Raymond D., Portland, Oregon
 Manion, William C., Washington, D. C.
 McMullen, Thomas, Des Moines, Iowa
 McQuown, Albert L., New Orleans, Louisiana
 Reals, William J., Omaha, Nebraska
 Richfield, Daniel F., Cincinnati, Ohio
 Sinclair, Cecil L., New Orleans, Louisiana
 Swihart, John J., South Bend, Indiana
 Thayer, John E., Hartford, Connecticut
 Truemner, Keith M., Detroit, Michigan
 Tucker, Francis C., Chicago, Illinois
 Wallace, John L., Jr., Ft. Worth, Texas
 Whitaker, John L., Tacoma, Washington

AMERICAN SOCIETY FOR THE STUDY OF ARTERIOSCLEROSIS

The second meeting of the American Society for the Study of Arteriosclerosis was held at the Hotel Knickerbocker, Chicago, Sunday and Monday, November 2 and 3, 1947. The sessions on November 2 were concerned with "Anatomic Considerations in the Study of Arteriosclerosis" and "Physiologic Considerations in the Study of Arteriosclerosis". On November 3, papers on "Metabolic Considerations in the Study of Arteriosclerosis" and "Therapeutic Considerations in the Study of Arteriosclerosis" were presented. A banquet was held Sunday evening at which Dr. R. A. Katz spoke on "Arteriosclerosis in Europe; A Report" and Professor Hans Selye, of Montreal, spoke on "Hormonal Factors in Hypertension".

COURSES IN THE LABORATORY DIAGNOSIS OF PARASITIC DISEASES

Three refresher courses in the Laboratory Diagnosis of Parasitic Diseases will be offered for laboratory personnel during 1948 by the Laboratory Division of the Communicable Disease Center of the United States Public Health Service. The inclusive dates for these courses are: January 12 to February 20, 1948; July 12 to August 20, 1948; and October 11 to November 19, 1948.

Laboratory directors and senior staff members wishing to attend any of the six-week courses may do so. However, it is proposed also to schedule one or two short two-week courses for such persons. Definite dates for these classes have not yet been set. Those interested in attending the two-week courses should notify Dr. R. F. Reider, Assistant Chief Laboratory Division, United States Public Health Service, Communicable Disease Center, 291 Peachtree Street, Atlanta, Georgia, as to which of the following proposed dates would be most suitable, stating first and second choice: March 8 to March 19, 1948; May 10 to May 21, 1948; and December 6 to December 17, 1948.

TECHNICAL SECTION

A CLINICAL VISCOMETER*

FRANK D. MANN, M.D.

From the Division of Clinical Laboratories, Mayo Clinic, Rochester, Minnesota

There are a number of reasons for considering the measurement of viscosity of plasma as being of clinical value. Viscosity is as fundamental a property of the plasma as is density. Whereas variations in density are due mainly to changes in total content of protein, variations in viscosity mainly reflect qualitative changes in protein. These qualitative changes are now studied by variable and nonreproducible procedures, such as the sedimentation rate, or by methods, such as electrophoresis and ultracentrifugation, which are quite restricted in availability. Houston, Harkness and Whittington⁴ have presented evidence of the clinical significance of plasma viscosity in tuberculosis and other diseases. They used a modified Ostwald type of viscometer. Probably most other clinical measurements of viscosity have been done with the Hess viscometer.

Without prolonged discussion, it may be stated that none of the numerous viscometers which has been described appears to us suitable for clinical measurements on plasma. At the Mayo Clinic we have designed an instrument rather similar to the original capillary viscometer of Poiseuille.¹ It consists of a small pipet to the lower end of which is fused a glass adapter to take a standard (Luer) needle (Fig. 1). A 22-gauge spinal needle, 7.5 cm. in length, serves as the capillary. The pipet is marked with three engraved lines: one just above the bulb, one just below the bulb and one a short distance above the attachment of the capillary. The viscometer is filled well above the upper mark, attached to its capillary and the fluid is allowed to fill the capillary. It is then placed in a small buret clamp, with the lower end extending into a tall cylindrical glass water bath. The viscometer is slid up in the clamp until the level of water in the bath just meets the lowest line on the pipet stem. During these operations a finger is, of course, kept over the opening of the pipet; the finger is then removed and the time of efflux between the marks above and below the bulb is measured with a stop watch. The fluid which flows into the bath does not appreciably change the water level. The effective pressure head thus changes only by the distance between the two marks on the bulb, usually 2 to 3 cm. The average of the initial and final pressures is a fair approximation of the average pressure head during the time of efflux, provided this average pressure has a value of 10 cm. of water or more. When the viscometer contains water, this pressure, P_w ,

in centimeters of water, will be given by the formula, $P_w = \frac{h_1 + h_2}{2} - h_3$, in which h_1 , h_2 and h_3 are the indicated distances in Figure 1, expressed in centimeters. When the viscometer contains a fluid, x , of specific gravity, d , the average pres-

* Received for publication, October 18, 1947.

sure head P_x , in centimeters of water according to the formula, will be $P_x = d_x \frac{(h_1 + h_2)}{2} - h_3$.

It is sufficiently accurate to consider plasma as having a constant specific gravity of 1.026; P_w and P_x will then be constant for any given viscometer. The fluidity, Φ_r , of any specimen of plasma or other fluid relative to water at the same temperature may be calculated as follows, provided Poiseuille's law holds: $\Phi_r = \frac{P_w T_w}{P_x T_x}$, in which T_w = time of efflux of water and T_x = time of efflux of specimen fluid. Constant temperature regulation is inconvenient because the necessary stirring interferes with the accurate setting of the level of the viscometer in the water bath. By use of a four liter glass cylinder the temperature was found to vary no more than 0.2 to 0.3 C. over a period of several hours. It therefore seemed best to determine relative fluidity at room temperature.

TABLE 1
TEST OF VALIDITY OF APPLICATION OF POISEUILLE'S LAW*

h_3 , CM.	P_w , CM. OF WATER	T_w , SECONDS	$P_w T_w$
34.9	10.4	211.0, 210.4, av. 210.7	2191
24.9	20.4	106.0, 106.0, av. 106.0	2162
10.1	35.2	61.0, 60.8, av. 60.9	2144

* The determinations in this table were made at a temperature of 25.3 C. The volume of efflux was 2 cc., the value for h_1 was 46.9 cm. and for h_2 , 43.6 cm., and the efflux times were measured with the viscometer set at three different levels in the bath, corresponding to the values indicated for h_3 . The abbreviations are those used in Figure 1 and in the text.

A table of values of $\frac{P_w T_w}{P_x}$ may readily be prepared for the limited temperature range used and Φ_r may be calculated by dividing the value for the observed temperature by the observed value for T_x . However, the viscometer should be frequently checked with water.

There appears to be general agreement² that the relative fluidity of the plasma is essentially independent of temperature over the range in which the protein is stable. Hence, the absolute fluidity may be obtained by multiplying the observed relative fluidity of plasma by the fluidity of water at the same temperature. Fluidity, as pointed out by Bingham and Roepke,² is more conveniently studied than its reciprocal, viscosity, because fluidity tends to vary in a linear manner. The temperature of the fluid in the viscometer bulb is not necessarily the same as that of the fluid in the capillary, although, at room temperature, it should not be greatly different. The temperature of the tiny amount of fluid entering the thin metal capillary should, however, almost instantaneously become the same as that of a standard thermometer placed near the capillary in the bath. This assumption was severely tested by comparing the efflux time of water initially at room temperature (25.6 C.) and initially at 4 C. When a

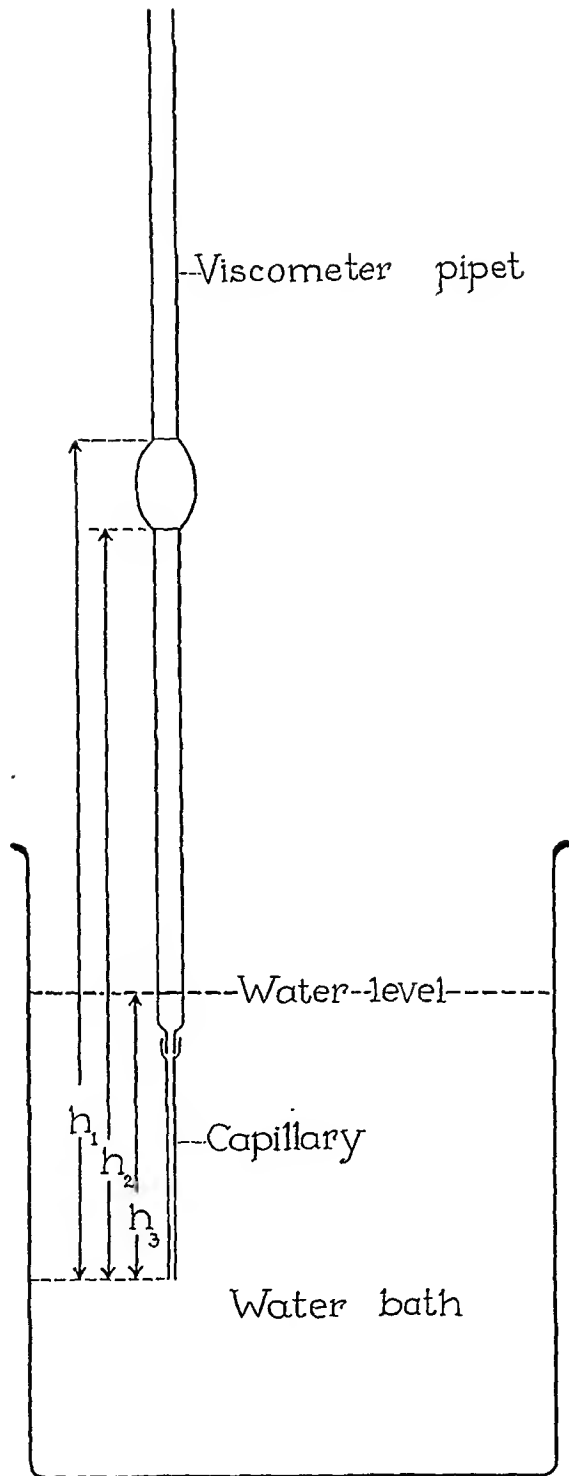


FIG. 1. Viscometer. The clamp, support and thermometer are not shown.

rate of flow several times that ordinarily employed was used, this difference of more than twenty degrees in initial temperature increased the efflux time from

61.0 to 62.8 seconds. Slight variations in the temperature of the bulb from that of the room, such as might occur incidentally, did not affect the efflux time.

Tests for conformity with Poiseuille's law are most conveniently carried out with this type of viscometer by varying the pressure head by setting the instrument at different levels in the bath. Such tests (Table 1) show satisfactorily constant values for PT with the approximate calculation of average pressure head as described and without correction for kinetic energy. Agreement of duplicate efflux times is also satisfactory. The dimensions of the viscometer are recorded; a relatively large instrument was chosen as an example in order to cover a considerable range of pressures and to include the maximal rate of flow studied. For clinical determinations on plasma, instruments with an average pressure head of about 10 cm. of water, a volume of efflux of about 0.5 cc., a total working volume of about 1 cc. and an efflux time with water of about one minute were employed. A piece of glass tubing with an inside diameter of about 2 mm. is suitable for such a viscometer.

From a practical point of view, the most important single source of error in any capillary viscometer is obstruction of the capillary, since particles undetectable by any other means may appreciably affect the efflux time. This is especially important in measurements on plasma, which is obviously difficult to rid of all particulate matter. After each determination the viscometer pipet is detached from the capillary and rinsed with distilled water. Then the capillary is attached and the pipet, filled with distilled water, is forcibly emptied through the capillary. The pipet is then dried with acetone, but the capillary is merely blown out with air. By use of this simple and rapid cleaning routine, accurately reproducible efflux times for water at a given temperature are usually observed with a given viscometer, but occasionally these control determinations are found to be several seconds too high. This is presumably due to some slight obstruction of the capillary which is best eliminated by forcibly blowing water, then air, through it several times. If desired, the needle stylet may also be used. Obviously this unpredictable, although in our experience rare, occurrence may introduce an occasional error of the order of magnitude of about ± 5 per cent. Duplicate observations are no protection against such errors since very frequently the efflux time may be increased by about the same amount in several successive runs. Since no practical means of eliminating such an occasional error from routine determinations could be found, it is simply recognized as a limitation of the method, applying not only to this viscometer but to any capillary viscometer when the fluid tested is not completely free of dust.

By use of the viscometer just described, plasma fluidity was estimated on ninety-eight normal blood donors. Five cubic centimeters of blood were collected in graduated tubes containing dried oxalate (6 mg. of $[\text{NH}_4]_2 \text{C}_2\text{O}_4$ and 4 mg. of $\text{K}_2\text{C}_2\text{O}_4$). The blood was centrifuged at high speed for approximately twenty minutes. Care was taken not to disturb the cells in pipeting plasma into the viscometer. The average relative fluidity for the group was 0.555, the extreme range, 0.500 to 0.603. This is about the degree of variation one would expect in view of the normal variation in protein. It should be stressed, how-

ever, that viscometry does not give the same information as does the determination of total protein and albumin-globulin ratio, since fluidity is mainly affected by the larger and more asymmetric protein molecules, especially those of fibrinogen, which usually make up a small fraction of the total protein. The normal values observed by Houston and co-workers⁴ are lower than ours, since they diluted the plasma with citrate.

The most important advantage of this viscometer is ease of cleaning; the labor of cleaning most capillary viscometers is sufficient to preclude their routine clinical use. The working volume of fluid may be only 1 cc. and need not be exactly measured. The instrument is sufficiently accurate, easily used and inexpensive. It is believed that it will be suitable for clinical measurements.

SUMMARY

A viscometer for clinical use is described; with it, normal values for plasma fluidity lie between 0.500 and 0.603, with an average value of 0.555.

REFERENCES

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POSSIBLE SOURCE OF ERROR IN THE QUANTITATIVE DETERMINATION OF UROBILINOGEN BY WATSON'S METHOD*

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During quantitative determination of urinary urobilinogen in a number of patients it was observed that values below 0.2 mg. per twenty-four hours were so common that the reliability of our technic was suspected. In searching for sources of error it was noted that urobilinogen values during sunny days were usually low, while those determined on cloudy days were more nearly within the range of expectation. Since our laboratory is brightly lighted by direct daylight it was natural to suspect this factor.

METHODS

Aliquot portions of twenty-four hour specimens of urine were subjected to simultaneous quantitative determination of urobilinogen content (1) while being ordinarily exposed to varying intensities of direct, reflected or diffused sunlight subsequent to ferrous sulfate precipitation, and (2) after a modification of technic affording protection from daylight during the entire procedure. The analysis of each pair of aliquot portions was carried through promptly to completion before that of the next specimen was begun.

In addition, the urinary urobilinogen values obtained from 50 patients prior to the modification suggested above were compared with a like number determined subsequently. The patients in both groups were chronic alcoholics of comparable age and sex with no jaundice or clinical evidence of portal cirrhosis. Any difference found to exist between the two groups could therefore logically result from modification of the procedure.

RESULTS

The data from Table 1 indicate that direct, reflected or diffused sunlight passing through ordinary window glass is able to reduce the recovery of urobilinogen in rough proportion to the intensity of the light. A loss as high as 87 per cent was noted when the procedure subsequent to ferrous sulfate precipitation was carried through in bright sunlight falling through an unfrosted skylight. A lesser but still significant loss was observed on cloudy or dark days when the procedure was done under lighting conditions comparable to those doubtless existing in many laboratories illuminated wholly or in part by daylight.

Examination of the data in Table 2 reveals the urobilinogen values to be significantly higher when protection from sunlight is afforded throughout the entire procedure. In 50 specimens of urobilinogen which were not protected from daylight, 16 per cent of the values obtained were less than 0.2 mg. per

* Received for publication October 10, 1947.

twenty-four hours, the values ranging from 0.023 to 21.80 mg. with a mean of 1.71 mg. per twenty-four hours. In 50 specimens of urine which were protected from daylight, none of the values fell below 0.2 mg. per twenty-four hours, the values ranging from 0.20 to 33.55 mg. with a mean of 2.12 mg. per twenty-four hours.

TABLE 1

THE LOSS OF URINARY UROBILINOGEN IN ALIQUOT SAMPLES UNDER VARYING INTENSITIES OF DAYLIGHT WHEN THE PROCEDURE IS NOT MODIFIED TO AFFORD PROTECTION AGAINST SUNLIGHT

SPECIMEN	WEATHER	UROBILINOGEN IN MG. PER 24 HOURS		PER CENT LOSS OF UROBILINOGEN IN SPECIMEN NOT PROTECTED FROM SUNLIGHT
		In specimen protected from sunlight	In specimen not protected from sunlight	
1	Dark day, raining	2.70	2.29	15
2	Dull day, hazy	0.50	0.41	18
3	Dark day	0.673	0.426	36
4	Dull day, hazy	0.750	0.425	43
5	Dull day, hazy	0.27	0.13	52
6	Bright day	1.67	1.05	37
7	Bright day	3.69	2.00	46
8	Bright day	0.31	0.15	51
9	Bright day	1.49	0.60	59
10	Very bright day	27.74	10.49	62
11	Extremely bright day	4.54	0.56	87

TABLE 2

THE SIGNIFICANT INCREASE IN QUANTITATIVE URINARY UROBILINOGEN AFTER MODIFICATION OF THE PROCEDURE SO AS TO AFFORD PROTECTION FROM DAYLIGHT

SPECIMENS OF URINE	EXTREMES FOR 50 PATIENTS IN MG. PER 24 HOURS	MEAN FOR 50 PATIENTS IN MG. PER 24 HOURS	PER CENT OF 50 PATIENTS WITH VALUES BELOW 0.2 MG. PER 24 HOURS
Not protected from sunlight.....	0.023 to 21.80	1.71	16
Protected from sunlight.....	0.20 to 33.55	2.12	0

Watson's method (1) protects urobilinogen from the action of light during the process of ferrous sulfate precipitation (ferrous hydroxide reduction) but does not mention a similar precaution to be observed during the remainder of the procedure. It was apparently presumed that those working with the method should be sufficiently aware of the photosensitivity of urobilinogen to avoid such a source of error. Since this was not the case in our laboratory it was thought possible that a similar oversight might exist in the technic of others.

This method of determining urobilinogen promises to be of great popularity as a test of liver function. It will therefore be of importance for comparable

results to be reported from various laboratories. In order to make such comparisons of greater accuracy and value it is suggested that those working with this technic determine whether the lighting conditions in their laboratories might cause a significant error in the determination.

The effect of exposure to artificial light was not determined.

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RAPID DETERMINATION OF UROBILINOGEN IN FECES*

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In 1944, Watson and his coworkers presented an abbreviation of the longer, more precise method for the quantitative determination of feces urobilinogen.^{1, 2} This modification should do much to popularize this important diagnostic test in clinical laboratories. We have developed a further modification of this method which obviates the time-consuming and rather unpleasant task of braying and extracting the fecal sample with a mortar and pestle. It has been our experience that a technician can now do a complete feces urobilinogen determination in about ten minutes of actual working time.

METHOD

The carton containing the feces is weighed, the weight of the feces obtained by difference, and the specimen then thoroughly mixed. Ten Gm. of the feces is weighed out on a piece of filter paper 7 cm. in diameter. The paper containing the feces is placed in a Waring blender container together with about one-half of the 300 cc. of water with which the feces is to be ground and extracted. The sample is subjected to mixing in the blender for approximately five minutes and then decanted into a 1000 cc. Ehrlenmeyer flask which contains 100 cc. of 20 per cent ferrous sulfate. The Waring blender container is then washed out with the remaining water which in turn is decanted into the Ehrlenmeyer flask. If desired, the container may be further rinsed out with the 100 cc. portion of 10 per cent sodium hydroxide which is then added to the Ehrlenmeyer flask. The rest of the procedure is the same as outlined in Watson's method. At times we have found it necessary to centrifuge a small part of the reduced feces mixture for two or three minutes in order to obtain a clear supernatant fluid.

RESULTS

Table 1 shows the results obtained by treating 10 samples of feces by this method and the method of repeated grinding and extraction, both by the quantitative petroleum ether extraction method (results given as mg.) and by the rapid method modification (Ehrlich units). It is seen that the results obtained with the method employing the Waring blender are similar to the results obtained with the method of repeated grinding and extraction, being slightly higher in most cases but agreeing within 5 per cent on the average.

SUMMARY

A quantitative method of determining urobilinogen in feces, which substitutes

* Received for publication, September 22, 1947.

a Waring blender for mortar and pestle extraction of urobilinogen from the feces, is presented. The simplicity and speed of this method should make this determination one which can be more readily performed in many clinical laboratories.

TABLE 1
COMPARISON OF RESULTS OBTAINED BY WARING BLENDER METHOD AND
METHOD OF MANUAL GRINDING WITH MORTAR AND PESTLE

SPECIMEN NUMBER	UROBILINOGEN IN EHRlich UNITS PER 100 GM. FECES		UROBILINOGEN IN MG. PER 100 GM. FECES	
	Mortar and Pestle	Waring Blender	Mortar and Pestle	Waring Blender
1	105	162	76	107
2	150	160	131	135
3	350	340	225	222
4	125	111	86	89
5	290	300	235	265
6	200	170	160	125
7	260	285	170	198
8	90	90	64	61
9	99	109	81	86
10	195	195	140	140
Averages	186.4	192.2	136.9	142.9

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RAPID METHOD FOR COLLECTING DOG'S BLOOD*

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The method to be described is used routinely by veterinarians to collect blood samples from large animals such as horses, cattle and sheep. However, the procedure can be applied to collecting blood from dogs, and our laboratory has found it superior to other methods commonly used. Large quantities of blood or smaller hourly samples may be taken rapidly from the external jugular vein without much discomfort to the dog, or injury to the vein. The bleeding is done directly into a sterile container, thus eliminating many sources of contamination.

We have collected 170 cc. of blood, at one bleeding, from a 27 pound dog without injury to the animal. Two of our dogs have been bled several times a week for the past eleven months. Both animals have remained in good health during the entire period, and the only noticeable damage to the veins is a small amount of scar tissue at the site of the venipunctures. The hunting breeds, at least a year old and weighing between 25 and 40 pounds, have proved to be the most satisfactory animals for our work, but Boston bulls, German police, mongrel and other breeds have been used with good results. Regardless of breed, however, one should avoid using over-weight dogs as layers of fat make the vein difficult to locate.

PROCEDURE

For small amounts of blood, insert the distal end of a 15-gauge California bleeding needle (purchasable from veterinary supply houses) between the cotton plug and wall of a sterile 127 mm. x 15 mm. centrifuge tube. For larger quantities, insert the hub of the needle into the proximal end of a rubber tube, having an inside diameter of 3 mm. and a length of 200 mm. (8 inches), and insert a glass tube, with an inside diameter of 5 mm. and a length of 75 mm. (3 inches), into the distal end of the rubber tube. The glass tube is passed between the cotton plug and the wall of a 250 ml. centrifuge bottle.

The test dog is placed on a table in either a standing or a sitting position, with the front feet held securely by leather thongs. The area of the venipuncture is shaved and sterilized by scrubbing with a cotton pad which has been saturated with an antiseptic solution. No local anesthesia is necessary. The assistant dorsiflexes the dog's head toward the tail. The technician anchors the jugular vein by exerting pressure with his left thumb along the jugular furrow, which lies between the dog's chest and shoulder. The bleeding needle is held in the right hand, and when the external jugular vein becomes visible, the needle is inserted at an angle of 45 degrees with the vein, the bevel of the needle

* Received for publication, September 17, 1947.



FIG. 1. BLEEDING POSITION
FIG. 2. BLEEDING FROM THE JUGULAR VEIN

being up. After the desired amount of blood has been collected, the pressure on the vein is released and the needle is withdrawn.

If the dog continues to bleed after the needle has been withdrawn from the vein, no attempt should be made to check the flow of blood as this may result in a large hematoma; the bleeding usually stops spontaneously within a few minutes. If a hematoma does develop, and further bleedings are necessary,

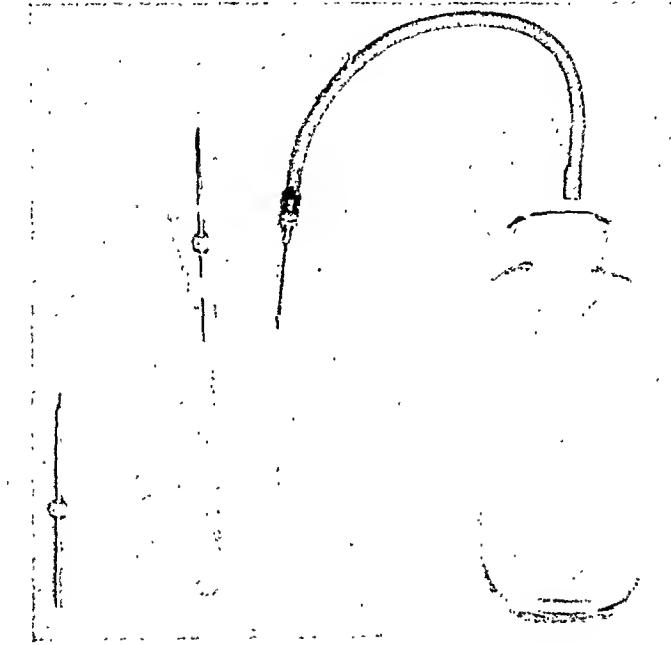


FIG. 3. BLEEDING EQUIPMENT

one can insert the needle into the external jugular on the opposite side of the neck, or into the vein above or below the hematoma.

SUMMARY

A method of collecting dog blood for biologic assays has been described, which we feel is superior to other methods commonly used. By this procedure, large or small quantities of blood can be collected rapidly, without elaborate equipment. No serious injuries to our test dogs have resulted from the use of this method to date.

THE CHANCO TECHNIC IN WRIGHT'S STAIN*

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The sudden onset of the war in the Pacific caught the Philippines with a limited supply of hematologic stains and conservation of the available material became imperative. Chanco,¹ after consideration of possible simplifications including the method of Field,³ therefore, introduced his modification of Wright's stain.

Chanco prepared his staining solution in a concentration of 0.5 per cent and, in order to prevent loss of staining material, used a Coplin jar containing the solution for immersion of the blood films, instead of flooding the slides in a horizontal position in the conventional manner. After immersion for twenty seconds in the stain, the slides were transferred to another Coplin jar containing ordinary tap water in which they were allowed to remain from thirty to sixty seconds. Then, while still in the jar, the slides were flushed with a strong current of tap water from the faucet to be sure all precipitate was removed. They were then dried and examined.

This technic was found quite satisfactory for general purposes and wastage was reduced materially. Thick films were treated in the same manner after dehemoglobinization with ordinary tap water. Red blood cells stained salmon pink, platelets purple, and the contrast stains of the white blood cells appeared well defined. Malaria parasites and trypanosomes (*T. evansi*) were readily identified and their contrast differentiation enhanced. In thicker smears, as of brain, liver, bone marrow and spleen, a longer period in the stain was necessary, but fairly satisfactory staining of the tissue elements was obtained. Chanco stated that the advantage of his technic was "its economy both in stain and methyl alcohol without sacrificing tinctorial efficiency. With the same amount of stain, ten to twelve times as many slides may be stained as with the usual Wright's technic."

The staining solution was kept at its optimum working efficiency by storage in a dark place when not in use and by keeping the top of the Coplin jar and the cover smeared with vaseline to reduce evaporation of the methyl alcohol. Weekly filtration was found desirable when the stain was used frequently. When the stain aged, which was indicated by poor definition of the white cell nuclei, the addition of from 5 to 10 cc. of methyl alcohol compensated for evaporation and tended to rejuvenate the stain. It was not found necessary to add any acid or alkali to improve its staining qualities. The solution produced good results for about two months. The presence of precipitates was found to be due to one or more of three causes: (1) the stain had not been filtered at regular intervals; (2) the stain had become too concentrated; or (3) the second Coplin jar had not been flushed properly with a strong stream of running tap water.

* Received for publication, September 8, 1947.

For the past fifteen months we have used Chanco's method in routine work and have found it to possess no disadvantage in comparison with the usual procedure of staining on slides or cover slips. Buffer solutions or distilled water may be used as desired to satisfy individual preference, but have not been found necessary in our experience. We have found the use of the Kahn shaker to mix and dissolve the powder in methyl alcohol, as recommended by Fredenburgh,⁴ to be a labor-saving procedure, especially since Chanco recommends a somewhat greater concentration of the stain than is generally used.

SUMMARY

The following technic for using Wright's stain, devised by Chanco, is recommended when speed, volume of output and economy are desirable in routine work.

1. Place slides in Coplin jar containing 0.5 per cent Wright's stain for twenty seconds.
2. Remove slides and place in second Coplin jar containing tap water for from thirty to sixty seconds.
3. Without removing slides from second Coplin jar, flush the jar with a strong current of tap water from a faucet for a few seconds.
4. Dry and examine.

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SIMPLIFIED METHOD FOR STAINING SPERMATOOA*

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Recognition of sterility in the male requires detailed study of seminal fluids. There is need for a simple method of removing coating mucus and other proteins and the staining of spermatozoa for easy differentiation of important morphologic aberrations. Many excellent, but difficult, stains for spermatozoa have been described, such as those of Cary and Hotchkiss,¹ Meaker,⁴ Gelarie,² Holbert,³ Williams *et al.*,⁶ Wollschwarz, and Pollak and Joel.⁵ The following simple method, which is a modification of Meaker's stain, has been found to give a clear picture of the morphology of the spermatozoa.

METHOD

One part of seminal fluid is shaken manually with one part of 5 per cent sodium bicarbonate for at least five minutes, and centrifuged for two to three minutes at 2000 to 3000 R.P.M. The supernatant liquid is poured off, the centrifugate washed in normal saline corresponding to the original volume and centrifuged again. The entire process of washing with normal saline is repeated. A few drops of saline are then added to the centrifugate and smears are prepared with a loop as for a bacterial stain. The films are dried at room temperature or preferably in an incubator (37 C.) and fixed by passing through a Bunsen or alcohol flame for two or three seconds. The slides are flushed with 95 per cent ethyl alcohol, drained and allowed to dry. A carbol fuchsin-95 per cent alcohol (1:1) stain is then applied for three minutes, the stain is washed off with water and a 1:3 aqueous methylene blue stain applied for two minutes. The slide is rinsed in running water for at least one minute and allowed to dry without blotting. The microscopic examination reveals the spermatozoal head-cap stained light blue, the nuclear posterior portion of the head dark blue, and the body and tail of the spermatozoa stained light red or pink, depending on the length of staining.

If thin even smears of sperm suspensions denuded of mucus are stained in the manner described above, important morphologic variations including aberrations of the acrosome and the body can be easily recognized. This stain, although simple and easily prepared, compares favorably with the more difficult and complex methods and is definitely superior to the commonly employed Gram's and Wright's stains.

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* Received for publication, September 29, 1947.

STANDARD TEST FOR STAPHYLOCOCCUS COAGULASE ACTIVITY*

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The coagulase test is commonly employed as an indication of the pathogenicity of staphylococci.^{4, 9} In carrying out studies on the nature of the reaction and its significance in infections, we were impressed with the lack of a standard procedure for performing this test. Suggested methods in the literature vary widely, and include the use of solid mediums^{1, 2, 5-7} and various proportions of plasma and broth cultures.^{3, 8} Since the concentrations of the reacting agents are of great importance, we have used the following method of serial dilutions of a standard broth culture.

MATERIALS

1. Human oxalated plasma, freshly drawn and handled aseptically, diluted 1:10 with physiologic saline solution.
2. Broth culture of *Staphylococcus*. A twelve-hour tryptose-phosphate broth, with para-aminobenzoic acid added.

METHOD

The test employs five tubes. To each of 0.5 cc. of serially prepared dilutions of the broth culture, representing dilutions of 1:10, 1:20, 1:40, 1:80 and 1:160, respectively, is added 0.5 cc. of the diluted plasma. The tubes are stoppered, placed in a water bath at 37 C. and read hourly for six hours. The reaction is read as 1 plus, 2 plus, 3 plus or 4 plus.

COMMENT

This method provides the range of optimum concentrations for the coagulase reaction. A known coagulase positive strain of *Staphylococcus* may fail to coagulate undiluted plasma or even plasma diluted 1:10, whereas with higher dilutions it may give strongly positive reactions. This phenomenon has not yet been fully explained, but it indicates the desirability of a standard test using serial dilutions, so that results from various laboratories may be comparable.

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* This study was aided by a grant from the Marshfield Clinic Research Foundation.
Received for publication, October 8, 1947.

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ACID-FAST PROPERTY OF *HISTOPLASMA CAPSULATUM**

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Parsons and Zarafonetis,¹ in his review of histoplasmosis, noted that *Histoplasma capsulatum* may be acid-fast. Since there is no single good stain for *H. capsulatum* in tissues, it was decided to find out whether the acid-fast property of the organism could be used diagnostically. Accordingly, microscopic sections from 8 cases of histoplasmosis were stained with carbol-fuchsin. At first the usual Ziehl-Neelsen method was used, but it was soon found that a larger number of organisms retained the carbol-fuchsin stain when the sections were decolorized with 3 per cent aqueous hydrochloric acid instead of with acid alcohol. The sections, which were still somewhat pink following decolorization, were lightly stained with methylene blue (thirty seconds), and then rapidly passed through 95 per cent alcohol and absolute alcohol before being placed in xylol. The results obtained with the modified Ziehl-Neelsen stain and with the Giemsa stain were as follows:

CASE NUMBER	SOURCE OF SECTION	RESULTS WITH MODIFIED ZIEHL-NEELEN STAIN	RESULTS WITH GIEMSA STAIN
45-979	Hospital of University of Pennsylvania	good	good
47-45		good	good
84213		good	not stained
87528		not stained	good
100125	Army Institute of Pathology	good	poor
121022		good	good
121023		fair	good
147816		poor	not stained

The modified Ziehl-Neelsen stain therefore gave results comparable to those obtained with the Giemsa stain. It should be emphasized that only a variable fraction of the number of forms present were actually acid-fast.

SUMMARY

A modified Ziehl-Neelsen stain may be used to advantage in the search for *Histoplasma capsulatum* in tissues.

Acknowledgments. The author is indebted to Dr. Balduin Lucké and Dr. Robert F. Norris for suggestions in this study, and to the Army Institute of Pathology for furnishing material for staining.

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TECHNICAL SUGGESTIONS

SIMPLE METHOD FOR DIVIDING FIELD OF MICROSCOPIC OCULAR

The following is a simple and highly satisfactory method of dividing the field of the microscopic ocular with hairs almost as fine as silk, or of making a pointer.

After removing the eyepiece lens of an ocular, a small amount of fairly thick microscopic slide mounting material, such as Permount,* is daubed on each of two opposite sides of the circular disc of the ocular. With a teasing needle, a thin fiber of mounting material is drawn from one side to the other across the opening. Several such fibers may be used to divide the field, or after drying a few hours, one fiber may be cut and half of it removed, the other half remaining to serve as a fine pointer. The material may be removed at any time with xylol.

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IMPROVED METHOD FOR SEALING HANGING DROP COVERSIP PREPARATIONS

In the usual method for sealing coverslips in the making of hanging drop preparations vaseline is used, either from a jar or from a lead tube. This has many disadvantages. First, since vaseline is a grease, it is soluble only in organic solvents and is not soluble in water. Greases are difficult to remove in cleaning glassware. Second, if jars are used one usually transfers the grease by means of the fingertips and thus there is a tendency to contaminate objects handled subsequently. Third, if a lead tube is used, great care must be taken to prevent it from becoming mashed and allowing the grease to escape.

In this laboratory, these difficulties have been overcome by using surgical lubricating jelly. This jelly is transferred to an ordinary syringe which is fitted with a blunt needle. This greatly facilitates dispensing and, at the same time, provides a storage space which is not as fragile as the lead tube.

Department of Anatomy

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GENTIAN (CRYSTAL) VIOLET SOLUTION FOR GRAM STAIN

Dissolve 2 Gm. of oxalic acid in 880 ml. of water. Add 20 ml. of aniline (aniline oil) and shake to dissolve. Filter through ordinary filter paper or omit entirely. Dissolve 5 Gm. of gentian (crystal) violet in 100 ml. of 95 per cent alcohol. Mix the two solutions. It is then ready for use. This "Gram violet" stains gram-positive organisms well, does not stain any gram-negative organisms so far encountered; there is no precipitate and it has good keeping qualities.

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M. STARR NICHOLS, PH.D.

* Obtainable from Eimer and Amend, New York, New York.

METHOD OF READING WEAKLY POSITIVE RH REACTIONS

When time is not an essential factor, I have found the following technic helpful in reading weakly positive Rh reactions. The test is performed in a test tube in the usual manner. All doubtful or negative reactions are corked and allowed to lie over-night at room temperature so that a column of the mixture lies along the side of the tube. When ready to read, the tube is gently raised upright and clumps, if any, can be seen easily.

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GRACE LeBEAU HEDGES, M.T. (ASCP)

DETERMINATION OF SALICYLIC ACID IN BLOOD

The technic for determination of salicylic acid level in plasma (as developed by Brodie, Udenfried and Coburn: J. Pharm. and Exper. Therap., **80**: 114-117, 1944) may be simplified by substituting ether for ethylene dichloride and ferric chloride for ferric nitrate.

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INOYO KOJIMA, M.T. (ASCP)

STAIN FOR *BORRELIA RECURRENTIS*

I have found that *B. recurrentis*, the organism of relapsing fever, stands out more distinctly when it is treated with the Giemsa stain without previous fixation in methyl alcohol. As with the thick drop malarial stain, the slides are well dried and then stained for one hour with 2 drops of Giemsa's stain to 1 cc. of distilled water.

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MEDICINE'S NEW FRONTIERS*

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The centennial of organized medicine in the United States is an appropriate occasion not only to recount our accomplishments to date, but to examine the horizon for promising trends.

In the field of infectious diseases, sulfa drugs and antibiotics have produced a marked reduction in the mortality rate, notably among respiratory diseases. Pneumonia is no longer "the old man's friend". Science is on the march towards control of the virus infections and future discoveries in the field of antibiotics will make new agents available and greater control possible.

It must be admitted that no significant therapeutic advances have been made in the treatment of arthritic and rheumatic disorders that are comparable to agents like insulin for diabetes, liver for pernicious anemia and penicillin for syphilis. The most satisfactory treatment to date is represented by the improvement of the general hygiene of the body with attention to anemia, focal infection, sluggish elimination and control of weight where indicated. The use of vitamins, gold salts and vaccines for rheumatoid arthritis, in the absence of infection has proved generally disappointing and medicine is seeking new leads in this field.

Cancer kills approximately 185,000 persons a year in the United States. One-third of these deaths, or from 60,000 to 70,000, now may be prevented if newer diagnostic procedures, supplemented by improved surgical techniques and radium therapy, are applied in time to discover and eliminate the cancer before general body dissemination has occurred. Thousands of persons who have harbored the dread cancer are living healthy lives today because modern science has made possible the elimination of neoplastic growths while they are still localized. It should be emphasized, however, that neither surgery nor irradiation with x-rays or radium has given us any real insight into the fundamental nature of cancer. Basic knowledge is being gained in cancer research by experts in cell physiology, chemistry, physics, genetics and metabolism. Cancer is a biologic problem and as such should be studied from a much broader approach than was the case until comparatively recently. Teams of qualified investigators, such as the one at The Research Institute of The Lankenau Hospital, are daily studying life processes. It was only twenty-five years ago that Stanley Reimann, your President, started his work in the field of growth with a view to gaining more knowledge of the ultimate nature of cancer. When clearer insight into the mechanisms of normal growth is achieved man will have gained an important victory. With the collaboration of groups, such as the one at The Lankenau Hospital, in association with others throughout our land and those abroad, the cancer scourge must reveal its secrets.

* Presented at the Twenty-Sixth Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 29, 1947. Received for publication, November 10, 1947.

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While the atomic age was introduced to mankind by the detonation of two bombs in Japan, events of even greater importance, if less spectacular, may evolve from the scientific researches, one result of which was the development of the bomb. The Fourth International Cancer Research Congress, recently held in St. Louis, was made a significant occasion by the receipt of an announcement from the President of the United States that radioactive isotopes would be made available to scientists throughout the entire world. This means that the United States has offered to scientifically trained men and women regardless of nationality, creed or race, the opportunity to add to man's knowledge of himself. The Atomic Energy Commission has stated that radioactive isotopes, which have been described as the most important tools in the field of biologic and medical research since the creation of the microscope, may be made available in adequate quantity and at reasonable cost to qualified investigators. Imagine the addition to our knowledge of digestion and nutrition, for example, which will be gained from following atoms of carbon, nitrogen and sulphur, from the time they enter the body, interact with the digestive juices, pass through the walls of the gastrointestinal canal, and eventually reach the brain, heart and muscles. They may be traced to the various glands of the body. Their paths will be followed until their final sites of deposition or until they are excreted from the body. Some twenty isotopes, and these represent only the beginning of this development, are already available for distribution throughout the world, presaging a new era in medical science.

The degenerative disorders of the body have come into prominence with the remarkable increase in the average life span of man since the turn of the century. Degenerative diseases of the vascular system affecting the heart, brain, kidneys and extremities, exact a toll of more than 600,000 American lives yearly. This is several times the number destroyed by cancer. What can be done to control high blood pressure, heart disease, cerebral hemorrhage, thrombosis and embolism? New knowledge is rapidly being gained. Anticoagulants are saving lives which ten years ago would have been lost. The earliest lesions, which ultimately lead to arteriosclerosis, will be much more clearly understood as a result of the use of radioactive tracers, the tagged atoms. These will tell us more about the intermediary fat metabolism which certainly plays a great role, if not the most important role, in the early degenerative changes that lead to arterial diseases. Ultimate control of disorders of fat and cholesterol metabolism may produce a delay in the degenerative processes of the vascular system. It should be emphasized that a great many individuals who have had an occlusion of a branch of a coronary artery, or thrombosis of a cerebral artery, by altering their daily activity and by living within their physiologic means, have added years to their lives and increased their zest and joy in living.

Another field of expanding importance concerns those persons with psychiatric problems, who are now utilizing 57 per cent of the hospital beds of our nation. Where mental breakdown has been the result of infection, such as syphilis or certain viruses, the destruction of the invading germ has frequently returned psychiatric patients to normal activity. Where defective genes have produced

psychiatric problems in individuals, custodial care must be provided. Split personalities, manic and depressive states, emotional instabilities and numerous other mental disorders are being examined with greatly improved methods. Many of these methods are the result of field studies, laboratory procedures and experimental observations carried out during both World Wars.

Certainly new insight has been gained in knowledge of individual aptitudes. This has been applied to practical advantage by many industries for assigning personnel to positions fitting their capabilities, with gratifying results. Assignment of workers to tasks compatible to their aptitudes and interests makes for more harmonious living. Currently, one hears much about psychosomatic disorders; yet a review of medicine historically will reveal that it has long been known that man cannot be separated from his environment and that environmental factors may precipitate mental disorders. Renewal of interest in the psychosomatic aspects of medicine has furnished knowledge of practical value and may cause the term "psychosomatic" itself ultimately to fall into disuse.

Social medicine is another aspect of medicine which requires study. Indeed, a Chair of Social Medicine has been created at Oxford, and is now occupied by John A. Ryle, a distinguished gastroenterologist. Renewed emphasis on the study of the environment should win the attention of the profession to renewed respect for external forces and the effects they produce within the human body. These environmental effects frequently produce discord within families and communities. Ultimately the injustices of community life may be traced to discordant reactions within the individuals who make up the community. A vicious cycle is thus set up and there must be an understanding of its underlying etiology if the individual is to be returned to a state of wholesome balance.

So today in the atomic age, medicine has a splendid opportunity to lead mankind to a better way of life. The creation of practically infinite quantities of physical energy should lead to the production of material goods which all the world needs. With the use of radioactive isotopes a clearer understanding of the basic processes of human life will be gained. With a better knowledge of man himself a more wholesome and more stable society should emerge.

EFFECT OF URETHANE ON MALIGNANT DISEASES

CLINICAL, HEMATOLOGIC AND HISTOLOGIC OBSERVATIONS ON PATIENTS WITH CARCINOMA, LEUKEMIA AND RELATED DISEASES*

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Recent reports have stimulated interest in the use of ethyl carbamate (urethane) and its derivatives for the treatment of malignant neoplastic diseases in man. Inhibitory effects on the course of the diseases have been reported,^{1, 5, 8, 10, 11, 12, 13, 19, 21, 24, 26, 27} but there is a dearth of detailed information on the changes in the blood, bone marrow and other tissues. We shall review the literature and present clinical, hematologic and histologic observations on 8 patients with various types of leukemia and other neoplastic conditions.

The literature contains reports of the use of urethane or closely related compounds in the treatment of 90 patients with neoplastic diseases. Paterson and coworkers²¹ reported on 32 cases of neoplasia including leukemia of both myelogenous and lymphatic type. Brief references to urethane therapy of leukemia have been made by a number of other writers.^{5, 11, 13, 19, 24, 26, 27} Heilmeyer¹⁰ reported on the effect of urethane in a case of Hodgkin's disease; Goodman and Lewis,⁸ in a case of metastatic carcinomatosis of the skin and Huggins, Yü and Jones,¹² in the treatment of carcinoma of the prostate. There are no descriptions of the findings in the bone marrow or other tissues, nor are the data on the blood changes complete.

SUBJECTS TREATED AND METHODS

We observed 8 adults and a 2 year old child in whom the clinical diagnoses were as follows: chronic myelogenous leukemia (2 cases) and chronic lymphatic leukemia, terminal subacute lymphatic leukemia, lymphatic leukemic reticulo-endotheliosis, multiple myeloma, mycosis fungoides and carcinoma of the lung with skeletal metastases. The patient with chronic lymphatic leukemia also had three squamous cell carcinomas of the face.

The urethane was administered orally in concentrations of from 6 $\frac{2}{3}$ to 10 per cent aqueous solution, flavored with syrup of glycyrrhiza, in divided doses during the day or in a single dose before bedtime. The daily dose varied from 0.5 to 6.0 Gm. per day for the adults and from 0.75 to 1.5 Gm. per day for the child. The total doses, which varied from 26.5 to 221 Gm., were given over periods ranging from nine to fifty-four days. Frequent complete blood studies, occasional sternal marrow aspirations, biopsies of enlarged lymph nodes, liver and skin tumors were obtained before, during and after treatment. The patients

* Presented at the Twenty-Sixth Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 28, 1947. Received for publication, October 20, 1947.

were weighed regularly. In 2 patients serial x-ray studies were made to observe changes in skeletal tumors. Autopsy material was available in 1 case. Liver and kidney function tests were repeated frequently.

1. CLINICAL OBSERVATIONS

Tolerance and clinical toxic effects. Various investigators have given urethane orally in daily doses of from 3 to 6 Gm. for periods of from four days to over nine months, in total amounts varying from 8 to 800 Gm. In Paterson's series, nausea occasionally accompanied by vomiting was observed in 21, slight drowsiness was noted in 2, and diarrhea occurred in 2 patients. In only 1 patient was nausea severe enough to require withdrawal of the drug. Vomiting can be avoided by administering the drug per rectum.^{21, 24} Farmer⁷ used urethane as an antispasmodic in patients with bronchial asthma. A daily dose of 4 Gm. for four or five days was well tolerated. A patient treated by Goodman and Lewis⁸ received 3 Gm. per day for thirty days, followed by a maintenance dose of 2 Gm. per day. These authors considered urethane a nontoxic drug. No clinical manifestations of toxicity or intolerance to oral administration of urethane were mentioned by others using daily doses of from 3 to 6 Gm., and total doses of from 45 to 448 Gm.^{5, 11, 13, 19, 24, 26, 27}

Temporary dangerous drops in erythrocyte and leukocyte counts or aplastic anemia may occur after heavy dosage over a long time.²¹ Huggins, Yü and Jones reported a death from hepatic necrosis which they attributed to urethane in a patient with carcinoma of the prostate who had received 9 Gm. daily for thirty-three days. These authors stated that it is unsafe to continue the drug when nausea and vomiting occur. Heilmeyer recommended intravenous administration because of the appearance of nausea and vomiting when urethane is administered orally. He injected 10 cc. of a 20 per cent solution morning and evening, and gave up to 10 Gm. by this route with no side reactions other than slight drowsiness. Vidabaek reported that oral administration of urethane was followed by nausea which was not observed after intramuscular injections of the drug.

In our experience, patients varied greatly in their tolerance to orally administered urethane. Anorexia occurred in most patients regardless of whether the drug was given in divided doses during the day or in a single large dose in the evening. Two of our patients never had nausea and ate well throughout the period of study. One patient received a total of 42 Gm. before experiencing repeated episodes of nausea and vomiting severe enough to cause withdrawal of the drug. A patient who was given 6 Gm. of the drug daily before retiring complained of dizziness and a sense of instability if he left his bed at night. These effects were transient and did not appear during the daytime. We did not find evidence that nausea and vomiting, in themselves, were absolute indications for stopping treatment. Therapy was stopped only because the drug was refused by the patient. The nausea and vomiting occurred immediately after the drug was ingested. This fact, and reports of tolerance of intramuscular, intravenous, or rectal administration, led us to believe that the symptoms are due to local gastric irritation.

Constitutional effects of urethane therapy. Goodman and Lewis' patient was in good condition at the onset of therapy and remained so after his skin tumors had regressed. Heilmeyer reported the disappearance of fever in a patient with Hodgkin's disease after fourteen days of treatment. Hemmeler described deterioration of the general physical status of patients with leukemia in spite of hematologic improvement. Huggins, Yü and Jones observed relief from pain and improved sense of well-being in patients with carcinoma of the prostate. Kartagener noted the development of leukopenia with aggravation of constitutional symptoms in a patient with leukemia.

A change not previously mentioned in clinical reports is loss of weight, which we observed in some of our patients who had received urethane over long periods. While anorexia and the resultant decrease of food intake were undoubtedly contributing factors, the weight losses appeared to be greater than could be explained on that basis. The possibility exists that weight loss may be caused, at least in part, by urethane therapy. Some support for this view is given by the work of Engstrom, Kirschbaum and Mixer⁶ in experimental animals. These authors treated leukemic mice with urethane and found that within three days the animals lost as much as 7 Gm. from initial weights of 25 to 28 Gm. The factor of fluid loss has not been evaluated.

Ultimate clinical effects and prognosis. Palliative and inhibitory effects of urethane therapy have been stressed; there are no claims of permanent results. Among Paterson's 32 patients with leukemia, 15 were considerably improved, 9 were improved but in unsatisfactory condition and 8 died. Two patients with acute leukemia died within two months. The response in lymphatic leukemia was less favorable and more variable than in myeloid leukemia. Among 24 patients with other forms of malignant disease there was a temporary diminution in the sizes of the lesions. Early palliative or temporary effects in 29 additional cases have been reported by others.^{1, 5, 8, 10, 11, 12, 19, 24, 26, 27} The effect of urethane in leukemia is similar to that obtained by x-ray therapy.^{21, 22} Huggins, Yü and Jones stated that the only effective agent in ameliorating prostatic cancer when it has become androgen independent and wide-spread is ethyl carbamate.

We observed little or no clinical improvement in 3 of 4 patients with leukemia who exhibited hematologic responsiveness and decrease in the sizes of enlarged lymph nodes, spleen, or liver. Two patients died of their disease during treatment. One patient (Case 5, terminal subacute leukemia) showed marked clinical and hematologic improvement but expired later of a "heart attack". A patient with chronic lymphatic leukemia and carcinomas of the skin (Case 6), who exhibited a favorable response to urethane, died of coronary thrombosis. The cause of death in a patient with leukemic reticuloendotheliosis (Case 1) who responded to treatment, was not determined. The general status of the 3 remaining patients was poor to begin with and it remained so after treatment, although in Case 4 (chronic myelogenous leukemia) there was hematologic and clinical improvement. In a patient with multiple myeloma (Case 2) there was no change in the skeletal lesions.

Summary of clinical reports and observations. In many cases urethane has inhibitory effects in a variety of malignant diseases including leukemia, lymphoblastoma and carcinoma. The effects of therapy, however, are variable and, as yet, unpredictable in any given instance. The most constant results have been obtained in patients with chronic leukemia, particularly of the myeloid type. There are no reports of retardation of the progress of the acute or terminal leukemias. Most patients tolerate oral administration of urethane in daily doses of from 1 to 6 Gm. Nausea and occasional vomiting, may be obviated in some patients by administering the drug per rectum, intramuscularly, or intravenously. Further study concerning the importance of nausea or vomiting as an indication for stopping treatment is needed. Judging from our experience these symptoms do not constitute such an indication. Other clinical manifestations of toxicity are slight drowsiness or dizziness which are not of major importance. Weight loss, out of proportion to reduced food intake, may represent an important constitutional injury to patients receiving the drug. Hematologic improvement may be independent of the changes in the general status of the patient, as some individuals showing such improvement nevertheless deteriorated physically. Serious complications such as aplastic anemia and, possibly, necrosis of the liver may occur. The ultimate clinical value of the therapy, and the prognosis of treated patients are subjects requiring further study.

II. HEMATOLOGIC OBSERVATIONS

The changes in the peripheral blood of our patients are indicated in the case reports and charts.

Effect of urethane on normal leukocyte counts. In normal mice urethane produces a drop in the leukocyte counts, but the drop does not appear as rapidly as in leukemic mice similarly treated.⁶ In 6 of 11 reported cases of carcinoma of the breast, initially normal leukocyte counts were reduced during treatment. In 5, the reduction was moderate, the counts falling to from 40 to 70 per cent of the pretreatment levels and returning to normal on cessation of therapy. In 1 case the leukocyte count dropped from 7800 to 1200 per cu. mm.; within eight days after withdrawal of the drug the count had risen again to 2670 per cu. mm.²¹

In 1 patient (our Case 2) the initial count was 6400 per cu. mm. After 83 Gm. of urethane had been given, the count was 2000 (neutropenia), and sixty-two days after stopping treatment the count was restored to 8000 per cu. mm. A patient with carcinoma of the lung (Case 7) developed an infection during the course of treatment after he had received 96 Gm. of the drug. Urethane did not inhibit the leukocytic response to infection, as the count rose to 19,350 per cu. mm. (Chart 3).

Effect of urethane on total leukocyte counts in leukemia. Urethane sharply reduces the incidence of transplanted lymphatic leukemia in rats.²⁰ Within three days after onset of urethane therapy of transplanted or spontaneous mouse myelogenous leukemia the total leukocyte counts drop from 10,000 per cu. mm. to normal levels.⁶

Urethane usually causes a fall in the total leukocyte counts in patients with

chronic leukemia. It has been shown that the amount of urethane required to produce a fall of 20,000 in the leukocyte count in chronic myelogenous leukemia varies from 19 to 124 Gm.²¹ Paterson *et al.* found that in 6 of 19 cases of chronic myelogenous leukemia the response to urethane was inadequate. They also observed 2 patients with chronic lymphatic leukemia in whom there was no response. One of these previously had failed to respond to isopropylphenyl carbamate. The lowest total dose of urethane which lowered a leukemic count to normal was 8 Gm. in a patient with chronic myelogenous leukemia. Karta-gener reported a case of chronic lymphatic leukemia in which the patient had an original leukocyte count of 120,000 per cu. mm. which was reduced to 4000

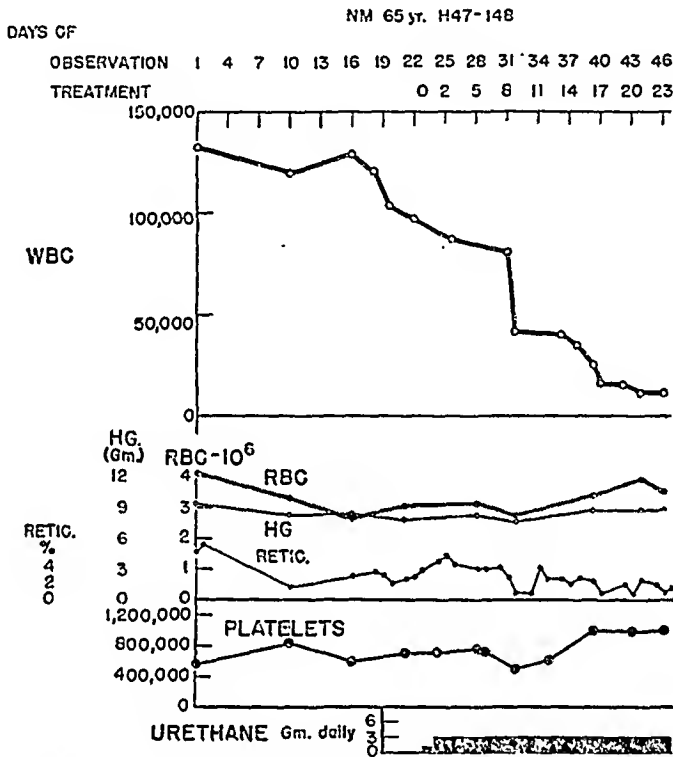


CHART 1. Case 3 (Chronic myelogenous leukemia).

with the appearance of absolute neutropenia. After stopping the drug, the count rose again to 21,000 but the neutropenia persisted.

Three of our patients experienced reductions in the total leukocyte counts as a result of treatment. In Case 3 (chronic myelogenous leukemia) the initial count of 133,800 per cu. mm. dropped to 9650 after 70 Gm. of urethane (Chart 1). In another similar case (Case 4) where the original count was 26,000, treatment was stopped when the count reached 9750. It was resumed because of an increasing leukocyte count, discomfort from an enlarged spleen and continued weight loss which we did not then evaluate as a possible complication of therapy. The drug was again withheld when the count was 1900 but there was a continuous gradual decline during the following fourteen days. This is suggestive of a continuing effect of urethane after withdrawal of the drug. There was marked

subjective improvement and gain in strength and appetite during the subsequent sixty-nine days without treatment. The leukocyte count in a patient with chronic lymphatic leukemia (Case 6) before treatment was 126,500 and 12,000 after fifty-five days of treatment with 221 Gm. of urethane (Chart 2). The patient refused further hospitalization and treatment was discontinued. During the following eighteen days the leukocyte count rose again to 29,450. A patient with lymphatic leukemic reticuloendotheliosis (Case 1) had an initial count of 8600 per cu. mm.; the count increased during treatment to 13,900 per cu.mm., but the rise was due to an increase in the absolute number of granulocytes, while stem cells and lymphocytes were becoming less numerous. Urethane had

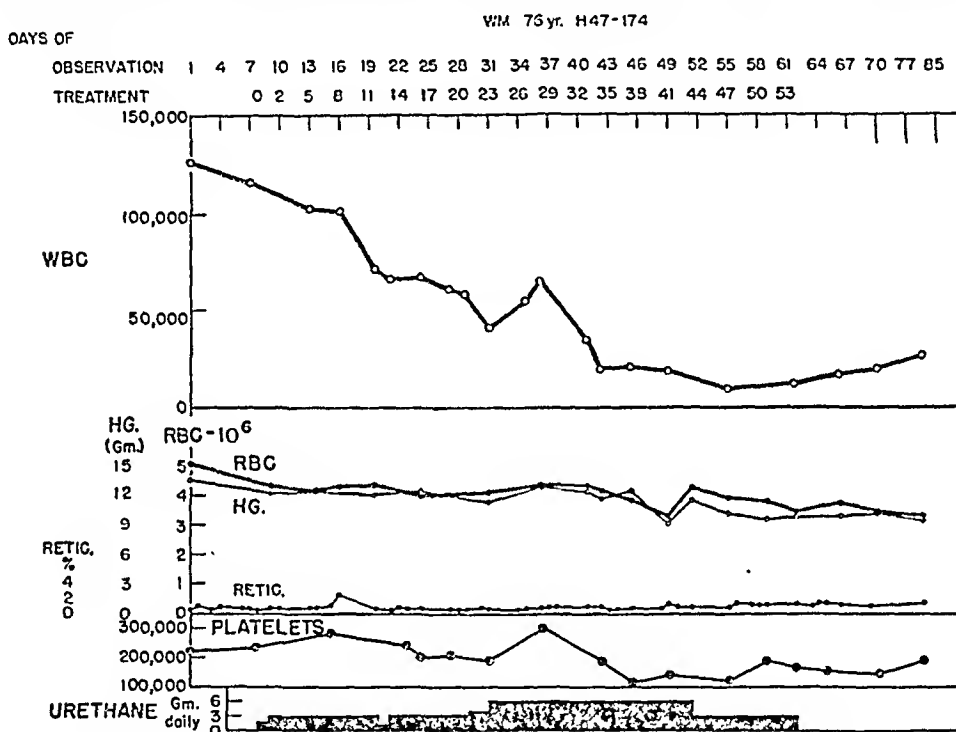


CHART 2. Case 6 (Chronic lymphatic leukemia).

no clinical or hematologic effect in a patient with terminal subacute lymphatic leukemia (Case 5).

Effect of urethane on the various types of leukocytes. In general, in responsive cases, the greatest reduction in percentage of circulating leukocytes reported is in the number of stem cell and promyelocytes. The more differentiated granulocytes appear relatively more resistant, and polymorphonuclear neutrophils appear most resistant. Although marked reduction in the absolute number of myeloblasts has been reported in acute myelogenous leukemia,²¹ Storti²⁶ saw no effect from urethane therapy in a terminal case with a myeloblastic blood picture and a count of 500,000 per cu. mm. We observed an increase in the absolute number of stem cells in our patient with terminal subacute leukemia (Chart 4).

Analysis of the hemogram by means of absolute counts of the various cell types

revealed that urethane may cause a disappearance or depression of the number of undifferentiated cells in leukemic reticuloendotheliosis, chronic lymphatic and myelogenous leukemia. A reduction of both myeloid and lymphoid cells was observed in some patients, whereas in others a reduction in the one occurred independently of a reduction in the other. For example, in Case 6 (chronic lymphatic leukemia) there was a disappearance of lymphoblasts, a marked reduction in the number of lymphocytes and a concomitant reduction in the absolute number of granulocytes (Chart 5). A similar effect was noted in a case of myelomatosis with an originally normal leukocyte count in which a temporary granulocytopenia and lymphopenia occurred. In patients with chronic myelogenous leukemia there was an orderly fall in the absolute numbers of granulocytes and their precursors, with little effect on lymphocytes (Chart 6).

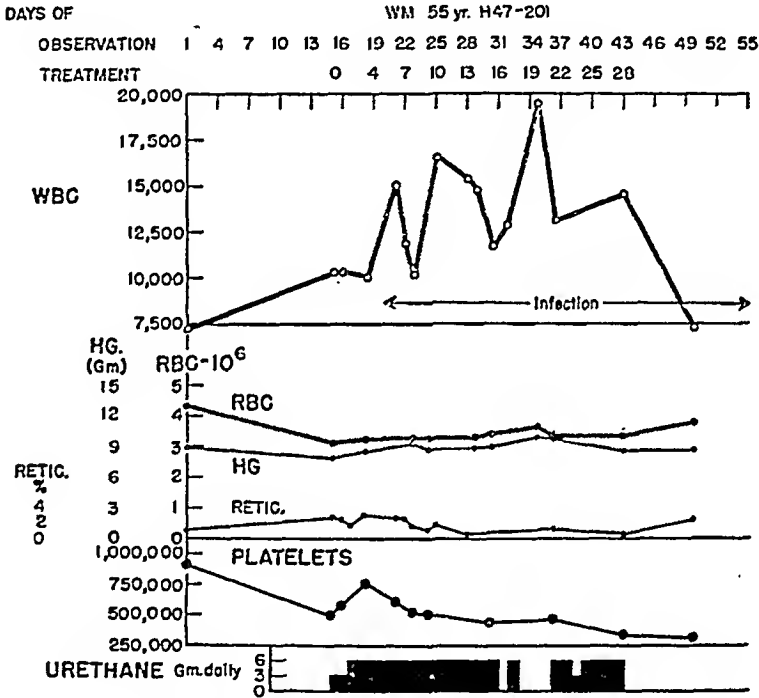


CHART 3. Case 7 (Carcinoma of lung).

In Case 1 (leukemic reticuloendotheliosis), stem cells disappeared and absolute lymphopenia developed in spite of a rise in the absolute number of granulocytes. In a patient with carcinoma of the lung and an originally normal leukocyte count granulocytes increased while an alymphocytosis appeared.

Our own observations and those of others^{21, 26} reveal no correlation between the daily or total dose of urethane and the rate or extent of reduction of total leukocyte counts or absolute counts of various types of circulating cells in patients with leukemia or other diseases.

Effect of urethane on the platelet counts. We were able to find only two brief references to platelet counts in patients treated with urethane. Kartagener observed counts ranging between 40,000 and 66,000 per cu. mm. in his patient

with chronic lymphatic leukemia. There was no significant change in the levels during urethane therapy. Vidabaek observed a fall in the platelet count from 300,000 to 119,000 in 1 of 4 patients with leukemia following urethane administration.

The platelet counts were determined frequently in all of our patients. No significant changes were noted in 3 patients. In a patient with chronic lymphatic leukemia (Case 6), the platelet level changed from 220,000 to 120,000 although the leukocyte picture had become nearly normal (Chart 2). The pretreatment levels in Case 3 (chronic myelogenous leukemia) were 566,000 to 700,000 per cu. mm. Although urethane caused a drop in the leukocyte count from 133,800 to normal, the platelet counts continued to rise, ultimately reaching levels over 1,000,000 (Chart 1). In Case 4 (chronic myelogenous leukemia), in which severe neutropenia developed, there was also a fall in the platelet counts from 300,000 to 50,000 per cu. mm. The platelet count continued to fall and

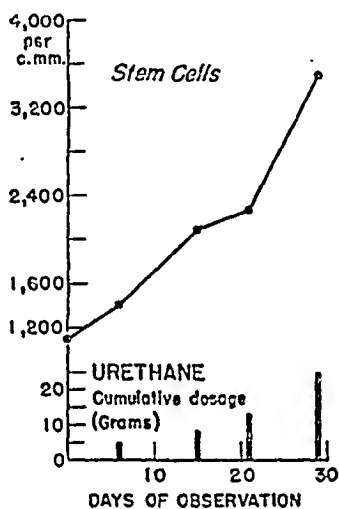


CHART 4. Case 5 (Subacute lymphatic leukemia). Absolute counts of stem cells in peripheral blood.

the erythrocyte and leukocyte counts reached very low anemic and leukopenic levels for a period after the drug was withheld. These changes indicated the appearance of a hypoplastic state of the bone marrow, as was shown later. The child with terminal subacute leukemia (Case 5) had platelet counts which fell from an initial level of 100,000 to 480 on the day of death.

Effect of urethane on erythrocyte, hemoglobin and reticulocyte levels. Paterson and coworkers reported only hemoglobin levels. In 13 patients with myelogenous leukemia the hemoglobin values rose in 10, remained stationary in 1, and fell in 2. They stated that patients receiving over 100 Gm. of urethane attained greater increases in hemoglobin values than those receiving less than 100 Gm. Kartagener observed that a normal erythrocyte count in his patient with leukemia remained unchanged after treatment with 84 Gm. of urethane. Storti and Schulze mentioned improvement of hemoglobin and erythrocyte levels

in persons with leukemia, anemia and chronic leukemia treated with the drug. Hemmeler found that anemia became worse in 2 of 6 individuals with leukemia. Rohr remarked that in patients with a relatively benign course the anemia improves, but that in cases with a severe course it becomes worse.

In our patients the hemoglobin and erythrocyte concentrations did not show independent fluctuations. The color indices remained fairly constant through-

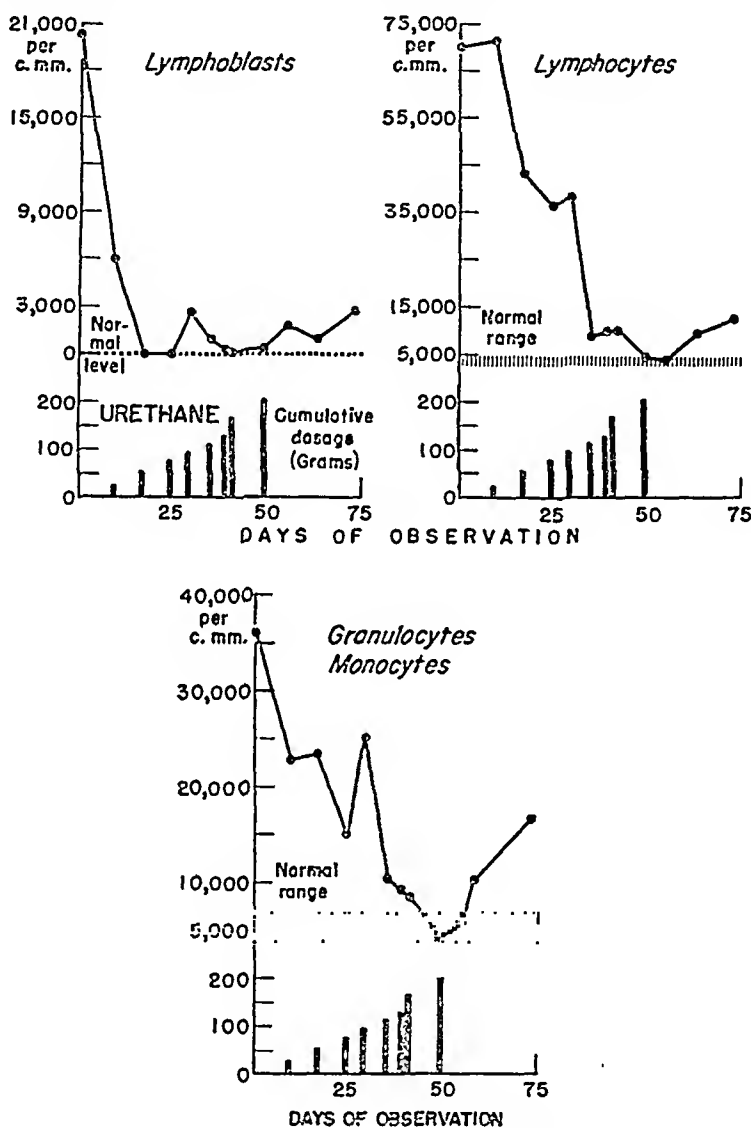


CHART 5. Case 6 (Chronic lymphatic leukemia). Absolute counts of cells in peripheral blood.

out treatment and there were no significant changes in the reticulocyte counts. In 5 patients with initial erythrocyte counts of from 2,090,000 to 5,150,000 per cu. mm. there were no appreciable changes after treatment. In Case 7 (carcinoma of the lung) there was a drop from 4,360,000 to 3,350,000 (Chart 3). This patient's extensive skeletal metastases increased in size during treatment. The drop in the count can be explained on the basis of progression of his disease. In Case 6 (chronic lymphatic leukemia) the count fell from 5,040,000 to 3,440,000

(Chart 2). Serial studies of the bone marrow in this patient revealed no decrease of the extent of leukemic involvement, in spite of a marked reduction in total leukocytes and disappearance of lymphoblasts.

Urethane may cause a serious, but reversible, fall in the erythrocyte count.²¹ This is illustrated in our Case 4 (chronic myelogenous leukemia) in which we believe that overdosage accounted for a change from an initial level of 4,060,000 to 2,670,000 per cu. mm. During sixty-nine days without treatment the red blood cell count rose again to 3,970,000.

The effect of urethane on the bone marrow. There have been very few studies of the bone marrow in animals or human beings receiving urethane. The drug causes a decrease in the number of cells and mitoses in the bone marrows of mice with myelogenous leukemia.¹⁶ On the other hand, Schulze found an increased incidence of mitotic figures among myeloblasts and myelocytes in the bone marrows of two patients with chronic myelogenous leukemia after urethane therapy. This occurred in spite of the usual fall in the total leukocyte count of the peripheral blood, and was considered to be indicative of fixation of mitoses, similar to the effects produced by colchicine, rather than evidence of accelerated leukopoiesis. Maringer observed that myeloid hyperplasia persisted in the marrow of a patient with chronic myelogenous leukemia after urethane had caused a change from a leukemic picture to a normal hemogram.

Marrow studies on our patients were carried out by means of sternal aspiration.² In one instance, postmortem specimens were obtained two hours after death. In a patient with multiple myeloma there were no changes in the marrow, as observed by sternal aspirations and serial x-ray studies, after administration of 83 Gm. of urethane. Since the marrow studies of other patients present varied reactions, the cases showing important changes are described below.

Case 1 (leukemic reticuloendotheliosis). The marrow sections appeared slightly hypocellular. There were scattered, poorly circumscribed masses of immature lymphoid cells, including hemopoietic reticulum cells which were identified in the smears. Myeloid cells were reduced in frequency (Fig. 1). After 53 Gm. of urethane had been given there was a relative increase in myelopoiesis. There were 4 per cent hemopoietic reticulum cells as compared with 12 per cent before treatment. Sections revealed a decrease in the size and frequency of leukemic nodules, and a definite increase in myeloid activity (Fig. 2).

Case 3 (chronic myelogenous leukemia). The pretreatment marrow sample was hypercellular and there was a slight left shift in granulopoiesis. The myeloid-erythroid ratio was greatly increased (50:1). After treatment and when the peripheral blood was approximately normal, there was not much change in the cellularity of the marrow or in the differential distribution of the leukocytes, although the myeloid-erythroid ratio had changed to 25:1, indicating a relative decrease in leukopoiesis during treatment.

Case 4 (chronic myelogenous leukemia). After 116 Gm. of urethane treatment the peripheral blood showed severe anemia, leukopenia and thrombocytopenia.

During the period of treatment, the marrow, which originally showed a relative myeloid leukocytic hyperplasia, became approximately normal in cellularity and cytology, although the peripheral leukocyte count was only 1900 per cu. mm. After treatment was stopped, the peripheral leukocyte, platelet and erythrocyte

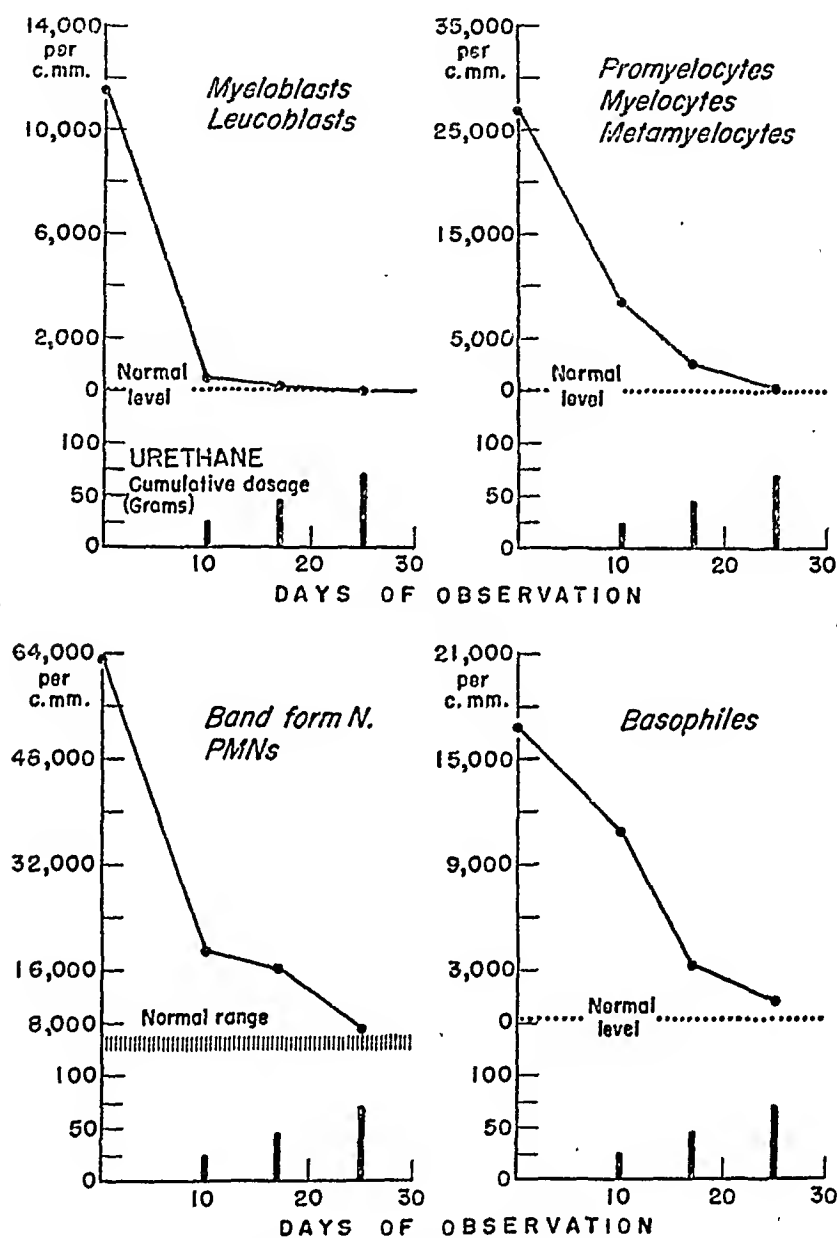


CHART 6. Case 3 (Chronic myelogenous leukemia). Absolute counts of cells in peripheral blood.

counts continued to fall. At the time these reached their lowest levels (eleven days after cessation of therapy) the bone marrow was extremely hypoplastic except for active megakaryocytopoiesis. The normoblasts, which were mainly of pronormoblast and basophilic types, were next most numerous, but except for band form and polymorphonuclear neutrophils there were practically no other myeloid leukocytes (Fig. 3). During the subsequent fifty-eight days numerous

sternal aspirations revealed the following sequence: the differential distribution of erythroblasts gradually became normal, with polychromatophilic forms predominating, a few myeloblasts and promyelocytes appeared, and megakaryocytopoiesis continued to increase to a point of hyperplasia (Fig. 4). The leukopoietic elements of the marrow were the most severely injured by urethane; megakaryocytes were least affected, and erythroblasts occupied an intermediate position in the scale of sensitivity.

Case 5 (terminal subacute leukemia). The pretreatment marrow smears contained over 90 per cent stem cells of indeterminate type. The second marrow sample was obtained at autopsy. The marrow of the sternum was similar to that of the ribs and vertebrae. There were many small hemorrhages which were also seen in other organs and tissues. The sections showed a generally hypocellular marrow with prominent reticulum and occasional cellular islands composed almost entirely of stem cells, many of which had pyknotic nuclei and showed karyorrhexis. There were scattered small foci of myeloid activity but megakaryocytes were rarely seen. The most obvious change was the appearance of large numbers of macrophages containing nuclear debris and occasional degenerating nuclei resembling those of the stem cells. Similar changes in other tissues were even more prominent and are illustrated in Figures 7, 8 and 9.

Case 6 (chronic lymphatic leukemia). The initial marrow sample was markedly hypercellular. There were 4 per cent lymphoblasts and 80 per cent lymphocytes; the remaining cells consisted of erythroblasts and granulocytes and rarely of megakaryocytes. After the leukocyte count had dropped from 126,500 to 12,000 per cu. mm. the second marrow sample was almost the same as that before treatment, except that lymphocytic replacement was even more extensive.

Summary of hematologic effects of urethane therapy. Urethane causes a fall in the absolute numbers of both lymphoid and myeloid leukocytes in the peripheral blood of patients with normal or leukemic leukocyte counts. The more undifferentiated the leukocytes, the greater the effect. Leukopenia, thrombocytopenia and anemia may result from over-treatment. These are reflections of bone marrow injury which may lead to temporary or permanent hypoplasia. Megakaryocytopoiesis persists longer than erythrocytopoiesis or leukopoiesis, and appears to recover earliest after urethane injury. The progressive effects of urethane may continue after treatment has been stopped.

Changes in the numbers of lymphoid and myeloid cells in the blood may occur concomitantly or independently. Even with doses large enough to affect the blood counts of leukemic individuals urethane may fail to inhibit neutrophilic response to infection. There is little correlation between dosage and extent or rate of change in the peripheral blood or bone marrow pattern. The results of therapy, in any given case, are irregular and must be observed by means of frequent blood and bone marrow studies.

No significant changes in reticulocyte percentages or color indices were observed in treated patients. Anemia, when present, may be favorably influenced in responsive cases, especially in chronic myelogenous leukemia, although in some patients anemia may become more severe during treatment.

The bone marrow has not been studied adequately. Leukemic infiltration or

replacement may or may not be influenced whether or not the blood picture tends to revert toward normal. In experimental animals urethane causes a reduction of the number of nucleated cells and mitoses in the bone marrow, although the incidence of mitoses may be increased after treatment in patients with chronic myelogenous leukemia. Dissolution of stem cells and phagocytosis of nuclear debris were extensive in the marrow of a patient with leukemia who failed to respond to urethane therapy.

III. OBSERVATIONS ON CHANGES IN ORGANS AND TISSUES

Effect of urethane on pathologic lymph nodes and spleen. Urethane greatly reduces the incidence of transplanted lymphosarcoma in rats²⁰ and causes a

TABLE 1
SIZE OF PRINCIPAL LYMPH NODE MASSES AND SPLEEN BEFORE AND AFTER URETHANE THERAPY

CASE	DIAGNOSIS	SIZE OF LYMPH NODES*		SIZE OF SPLEEN*		TOTAL DOSE OF URETHANE Gm.
		Before	After	Before	After	
1	Leukemic reticulo- endotheliosis	12.5	2.5	2.0	"smaller"	106.5
3	Chronic myelogen- ous leukemia			3.0	0.5	70
4	Chronic myelogen- ous leukemia			16.0	7.0	116
5	Terminal subacute leukemia	"en- larged"	"in- creased"	"enlarged"	"no change"	26.5
6	Chronic lymphatic leukemia	8.0	5.0	"Iliac crest"	10.0	221

* Numerical data represent maximum diameters of lymph node masses or distances between edge of spleen and costal margin in the left midclavicular line, in centimeters.

decrease in the sizes of lymph nodes and spleen in transplanted and spontaneous mouse myelogenous leukemia.⁶ Similar effects on the spleen have been reported in patients with chronic myelogenous leukemia^{21, 24} and on lymph nodes and spleen in patients with chronic lymphatic leukemia, lymphosarcoma, and Hodgkin's disease.^{10, 13, 21} The effects are often transient, the nodes becoming smaller but not disappearing entirely.

The changes in the sizes of lymph nodes and spleen in our patients are shown in Table 1. Histologic material from lymph nodes was available in 2 cases. The pretreatment lymph node section in Case 1 (leukemic reticuloendotheliosis) showed obliteration of normal architecture by cellular tissue composed of lymphoid cells with uniform nuclei slightly larger and more vesicular than those of lymphocytes. Occasional very large undifferentiated reticulum cells and forms intermediate between them and the predominating lymphoid cells were found.

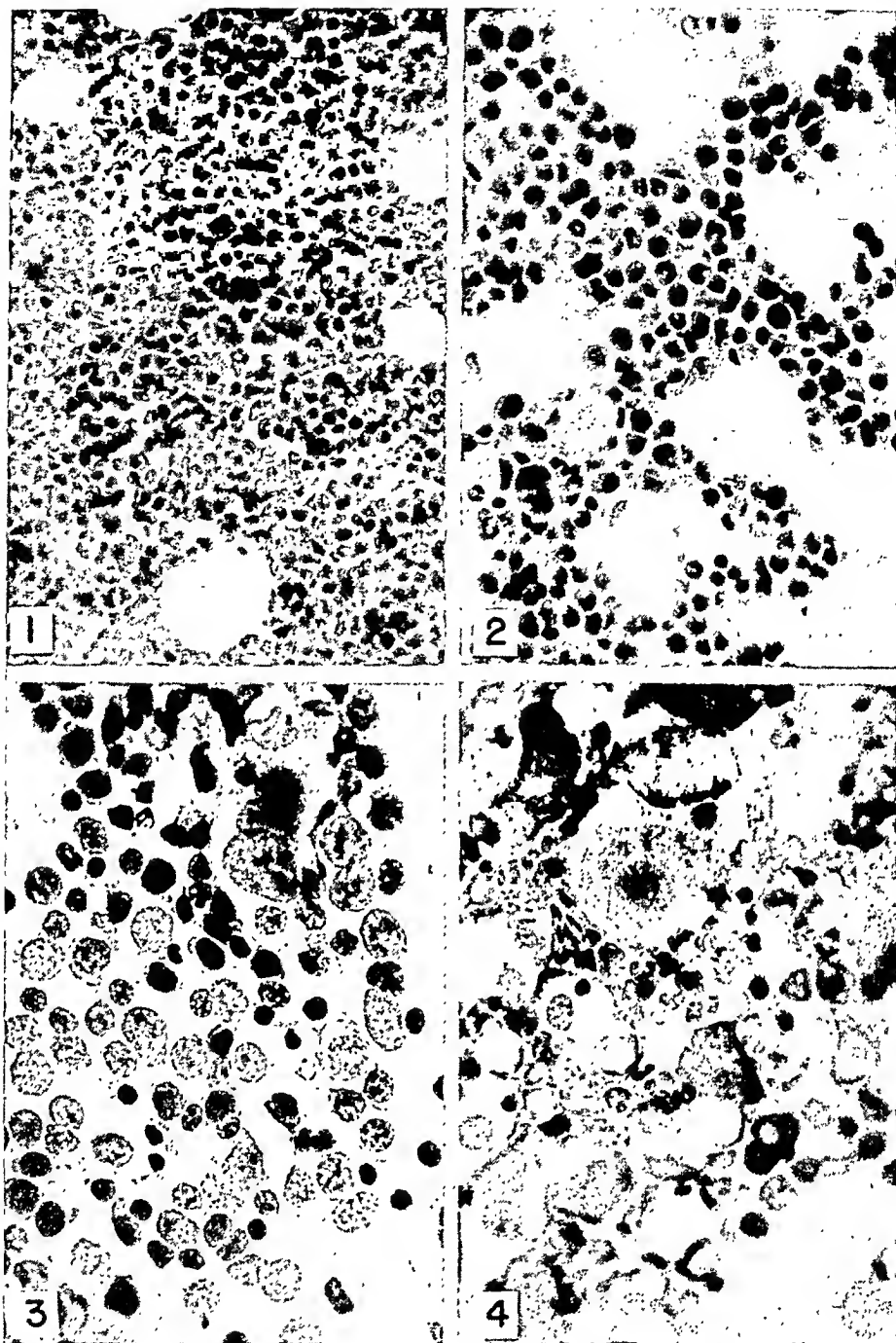
There were many mitoses. There was no fibrosis or hemosiderosis. An occasional rare phagocyte was found (Fig. 5). After treatment with 75 Gm. of urethane, and after definite softening and decrease in the size of the lymph node masses were observed, a second biopsy specimen was obtained which showed numerous areas of irregular replacement of lymphoid tissue by collagenous connective tissue, and many areas in which the structure of the tissue was loose and hypocellular. Mitoses were rare. Capillary dilatation, pyknosis of nuclei, karyorrhexis, and infiltration with hemosiderin-filled macrophages were prominent in the juxta-hilar portions of the node (Fig. 6). The changes were indicative of previous hemorrhage and dissolution of the leukemic cells.

The lymph node material in Case 5 (terminal subacute leukemia) was obtained two hours after death. There were striking changes in the leukemic foci of the lymphoid structures of lymph nodes, spleen, thymus and intestinal tract. The description of these findings in a lymph node applies to the leukemic foci of other organs including those of the liver, kidneys, pancreas, ovaries, bone marrow and choroid plexus. The leukemic foci in all sites were composed of large undifferentiated lymphoid stem cells. The important changes consisted of marked pyknosis of nuclei, karyolysis and karyorrhexis among the leukemic cells. There were very large numbers of macrophages containing nuclear debris, and often whole cells or nuclei similar to those in the intact leukemic foci (Figs. 7, 8 and 9). The changes, representing dissolution of the primitive lymphoid cells, are similar to those described in lymphoid tissues as a result of injection of pituitary adrenotropic or adrenal cortical hormones, or to exposure to a variety of toxic chemical agents, x-rays, radium, as well as environmental stresses such as cold or heat, or inanition which Dougherty and White⁴ ascribed to adrenal cortical stimulation, and which Kindred¹⁴ described as direct effects of roentgen ray irradiation or of the mustard amines.

Effect of urethane on the liver of patients with leukemia. We were interested in studying the liver by means of successive biopsies because of its frequent involvement in leukemia and the reported hepatotoxic effects of urethane. In rats urethane causes damage of the portal and sublobular veins and of the endothelial lining of the sinusoids²³ leading to extravasation of plasma and formed elements of the blood through the vessel walls. The changes are limited to the regions surrounding the portal areas.³ Sollman²⁵ stated that large doses of urethane cause vacuolar degeneration of the hepatic cells in rabbits.

Specimens of the liver were obtained for biopsy from 3 of our patients before and after urethane therapy. We were unable to find morphologic evidence of hepatocellular damage in these specimens or in an additional specimen obtained at autopsy. In one case of chronic myelogenous leukemia and in another of chronic lymphatic leukemia there were no changes in the leukemic infiltrations in spite of marked changes in the peripheral blood.

The liver tissue from the patient with leukemic reticuloendotheliosis, on biopsy, showed alterations of special interest. The pretreatment specimen was obtained peritoneoscopically. It was small, consisting mainly of a leukemic focus in a portal area and the surrounding hepatic parenchyma both of which were densely



FIGS. 1-4

FIG. 1. Case 1. Bone marrow section before treatment. There is replacement of myeloid tissue by irregular, poorly circumscribed masses of primitive reticular lymphoid cells.

FIG. 2. Case 1. Bone marrow section after the patient had received a total of 53 Gm. of urethane. There is active myelopoiesis.

FIG. 3. Case 4. Bone marrow smear. The marrow is predominantly erythroblastic. There are numerous basophilic normoblasts and very few leukocytes. The smear was obtained after the patient had received a total of 166 Gm. of urethane, and eleven days after cessation of therapy.

FIG. 4. Bone marrow smear obtained sixty-nine days after cessation of therapy. There has been a resumption of leukogenesis and a marked increase in the incidence of megakaryocytes.

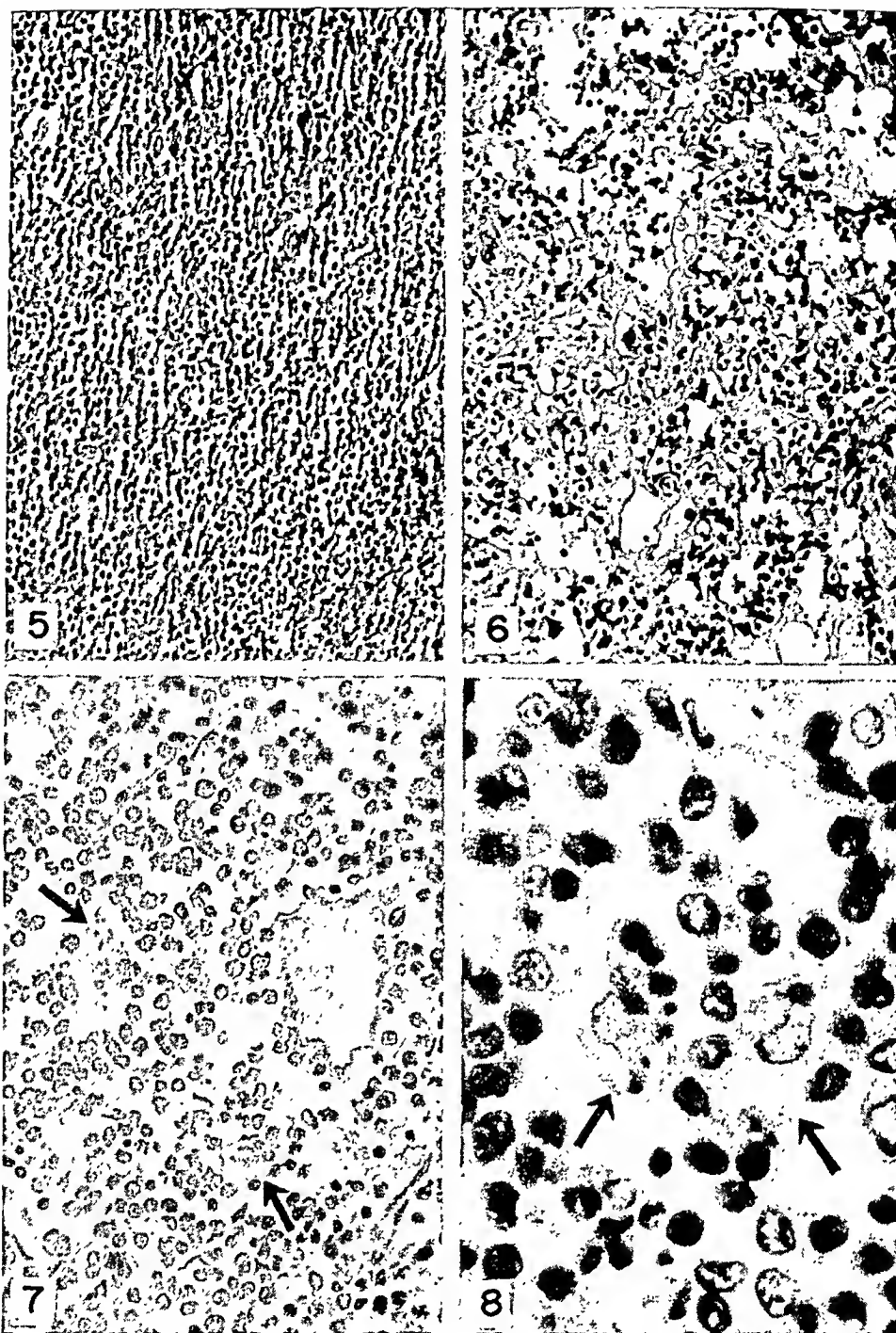
infiltrated with leukemic cells. Mitoses were numerous (Fig. 10). The second specimen, obtained surgically after administration of a total of 72 Gm. of urethane, consisted of approximately 30 or 40 hepatic lobules. Only one portal space contained a definite but small mass of leukemic cells similar to those seen in the earlier specimen. There were no mitoses. In nearly all portal areas there were masses of collagenous tissue, often of very loose structure in which many macrophages containing hemosiderin were aligned about the peripheries of the portal areas. Scattered isolated leukemic cells were sometimes present. In some of the portal spaces distended capillaries were seen, but there was no other evidence of vascular or parenchymal damage. The connective tissue masses were unlike those seen in portal cirrhosis of the liver, and there was no atrophy of hepatic epithelium (Figs. 11 and 12). The peculiar collections of loose, relatively acellular collagenous tissue had a distribution which is expected for leukemic foci, although few of the leukemic cells remained. The arrangement of hemosiderin-filled macrophages in the periportal regions recalls the locations of hemorrhages reported as resulting from urethane administration in rats. We regard the findings as suggestive of resolution of leukemic lesions, with superimposed mild vascular damage similar to that seen in the experimental animal.

Effect of urethane on other organs and tissues. There were no parenchymal changes which could be ascribed to urethane in the organs examined at autopsy in the case of the 2 year old girl who had received 26.5 Gm. of the drug. Absence of glomerular damage was of particular interest since urethane has been considered a capillary toxin^{17, 18} and is known to cause glomerular damage in certain strains of mice.¹⁵

The skin lesions in a patient with mycosis fungoides (Case 8) regressed rapidly under urethane therapy. Itching disappeared and the lesions became less hyperemic after 30 Gm. of urethane had been given. The improvement was much more rapid and of greater extent than the patient had previously obtained from x-ray therapy. We were unable to continue with treatment because of nausea and vomiting and the patient's refusal to remain hospitalized.

The patient with chronic lymphatic leukemia (Case 6) also had a large carcinoma of the skin over the right temporal region. The pretreatment and post-treatment appearances of the lesion are shown in Figures 15 and 16. During the first fourteen days of treatment a circular zone of hyperemia appeared in the skin around the edge of the tumor. Later, the central part of the tumor became necrotic and sloughed off. Necrosis, which involved the superficial one-half to two-thirds of the lesion, extended into the peripheral portions until nearly the entire surface appeared nonviable. In the meantime there was no lateral extension of the tumor. Although large squamous cell carcinomas of the skin are very likely to undergo degeneration in their central parts, especially after trauma or infection, the microscopic appearances of the tumor before and after treatment suggest that urethane may have had some effect on the lesion.

The pretreatment biopsy specimen from the edge of the tumor was a well-differentiated squamous cell carcinoma with marked cornification. The advancing margin of the tumor was composed of irregular masses of epithelial cells



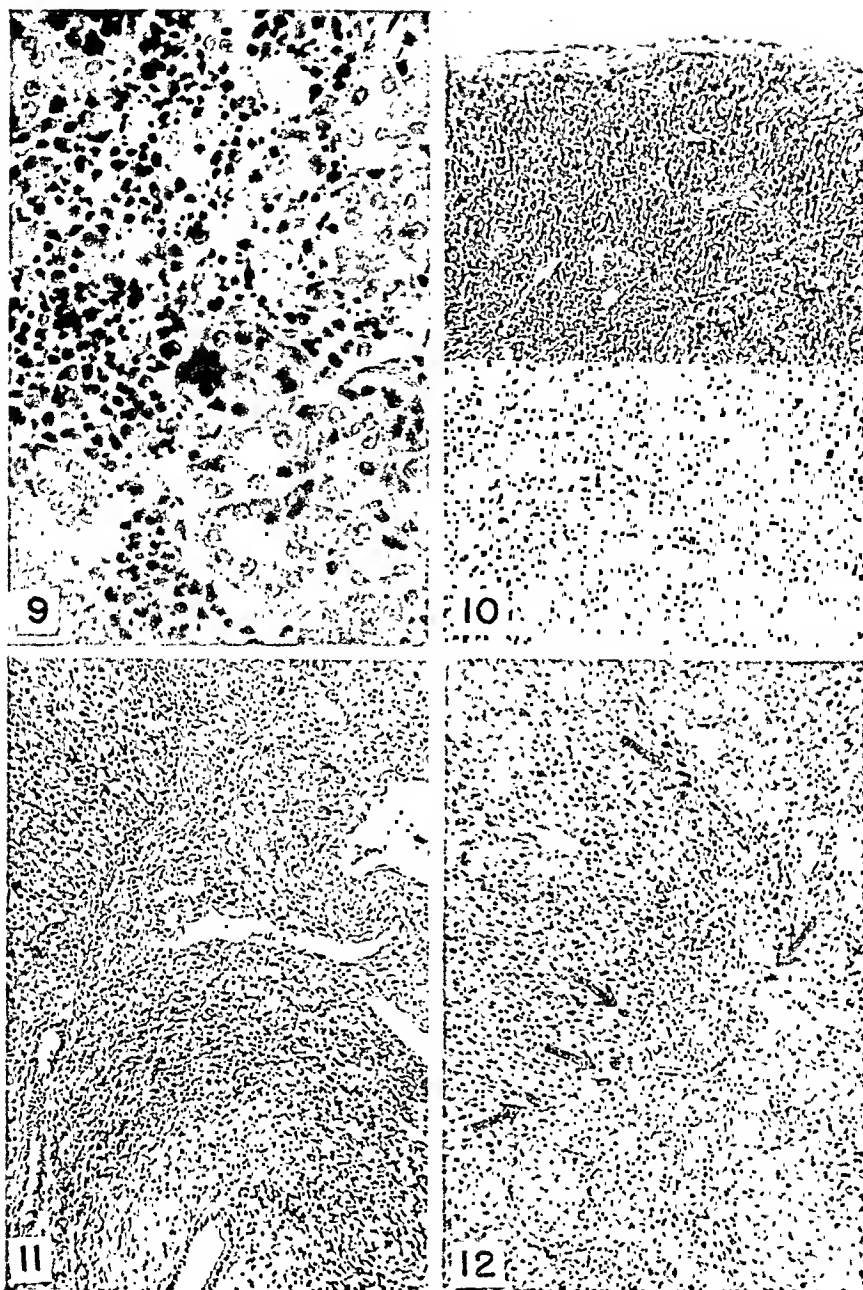
FIGS. 5-8

FIG. 5. Case 1. Section of axillary lymph node before treatment. The normal architecture is replaced by primitive reticular lymphoid cells.

FIG. 6. Case 1. Section of axillary lymph node after the patient had received a total of 75 Gm. of urethane. There is marked hypocellularity and replacement of tissue by loose, edematous collagenous connective tissue.

FIG. 7. Case 5. Section of axillary lymph node obtained at autopsy after the patient had received a total of 26.5 Gm. of urethane. The nuclei of many of the lymphoid cells are pyknotic. There are numerous macrophages containing nuclear debris (arrows).

FIG. 8. Case 5. High power photomicrograph from lymph node shown in Fig. 7. Macrophages contain nuclear debris (arrow).



FIGS. 9-12

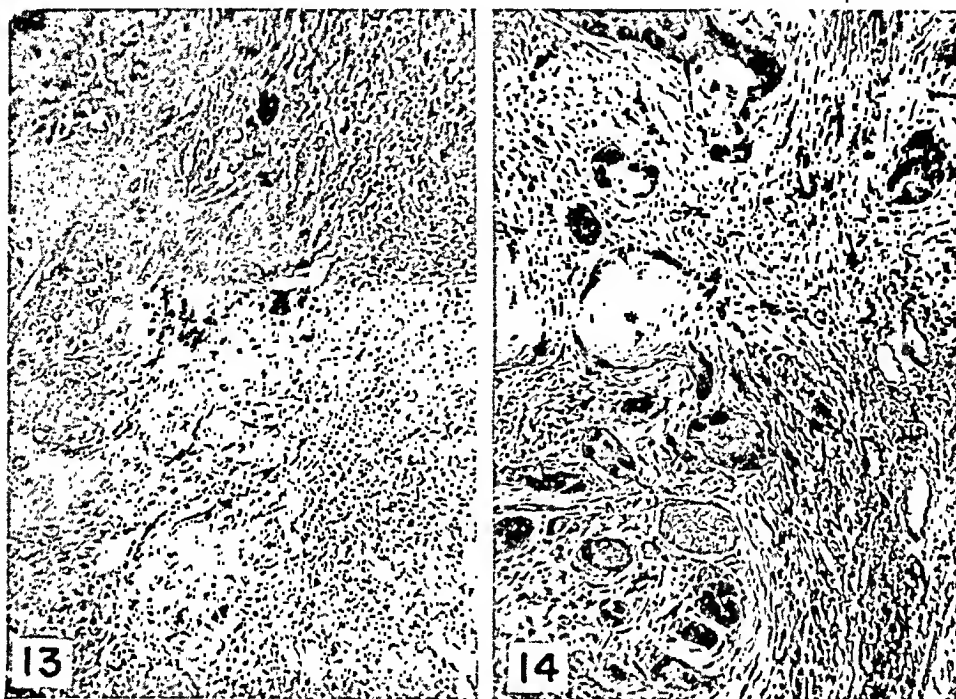
FIG. 9. Case 5. Section of pancreas obtained at autopsy after the patient had received a total of 26.5 Gm. of urethane. There is marked karyorrhexis among the cells of the leukemic infiltrations.

FIG. 10. Case 1. Section of liver before treatment. There is an extensive and very cellular leukemic mass in the portal connective tissue and adjacent hepatic parenchyma.

FIG. 11. Case 1. Section of liver after the patient had received a total of 72 Gm. of urethane. The portal areas are composed of loose, collagenous connective tissue with only small accumulations of leukemic cells. The hepatic cells are well preserved.

FIG. 12. Case 1. Another area in the specimen of liver tissue illustrated in Fig. 11. At the peripheries of the portal areas there are macrophages containing hemosiderin (arrows).

in squamous arrangement with relatively less cornification. The tumor infiltrated and replaced the connective tissue of the derma. There was little evidence of reaction in the adjacent connective tissue which was infiltrated with small lymphocytes (Fig. 13). There were a few small leukemic nodules in the outer portions of the derma beneath the normal squamous epithelium. The second specimen was obtained after 221 Gm. of urethane had been given. The advancing margin of the tumor was broken up into irregular small masses of epithelium separated by abundant newly formed connective tissue stroma. The stromal reaction extended slightly beyond the edge of the tumor (Fig. 14).



FIGS. 13-14

FIG. 13. Case 6. Section of squamous cell carcinoma of skin before treatment.

FIG. 14. Case 6. Section of squamous cell carcinoma of skin after the patient had received a total of 221 Gm. of urethane. The advancing margin of the tumor is broken up into small aggregates of tumor cells separated by abundant newly formed fibroblastic stroma.

The change in the histologic appearance characterized by the development of abundant recently formed fibroblastic stroma appeared unusual for this type of tumor, and it is similar to the change produced by urethane in the structure of the Walker rat carcinoma. Haddow and Sexton⁹ reported that under the influence of urethane the characteristic cellular texture of the Walker carcinoma gave place to a more fibrous structure with spindle cells and a distinctly more abundant stroma. They felt that the change was due in part to involvement of vessels and stroma. For these reasons we consider the similar alteration in the squamous cell carcinoma of the skin a possible effect of urethane.

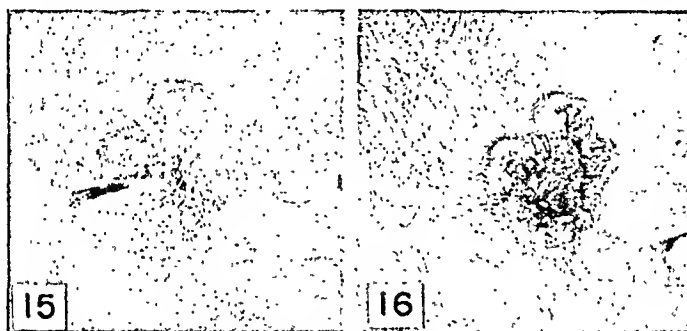
Summary of observations of effects of urethane on tissues. Urethane reduces the incidence of transplanted lymphosarcoma in animals and causes regression but

not complete disappearance of enlarged lymph nodes and spleens in leukemias of animals or man.

Leukemic foci in various organs may be reduced in extent. The histologic appearance of such phenomena is characterized by a reduction in the number of leukemic cells and mitoses, pyknosis of nuclei, karyolysis and karyorrhexis, phagocytosis of nuclear remnants and replacement by collagenous connective tissue. Some of these changes are similar to those reported as resulting from adrenal cortical stimulation, x-ray therapy or administration of mustard amines.

These effects are irregular and sometimes do not appear even though marked changes have occurred in the peripheral blood.

Temporary regression of other tumors including lymphoblastoma, mycosis fungoides, and carcinoma have been reported. The histologic changes in a squamous cell carcinoma of the skin subjected to urethane therapy included the development of a marked stromal reaction in the invaded tissue.



FIGS. 15-16

FIG. 15. Case 6. Squamous cell carcinoma of the skin over right temporal region before treatment. A recent surgical incision and a small superficial ulcer are present.

FIG. 16. Case 6. Squamous cell carcinoma of skin over right temporal region after the patient had received a total of 221 Gm. of urethane. There is extensive necrosis and sloughing.

Late effects of slight vascular damage in the liver and lymph nodes were found in 1 patient, but no damage was found on biopsy of the liver of 3 patients, and no hepatocellular or renal glomerular damage, or other parenchymal injury resulting from urethane therapy was found in the autopsy material of 1 patient.

DISCUSSION

A review of the literature and our own observations have convinced us that further investigation of urethane therapy of neoplastic diseases is warranted. The problems of dosage, route of administration and toxic effects have not been solved. In view of the variable results obtained frequent blood and bone marrow studies should be made to evaluate the effects of treatment and detect hematologic complications. Determination of absolute counts of leukocytes in the blood at frequent intervals, and of hemoglobin or erythrocyte levels at weekly intervals are probably adequate for these purposes. Unless complica-

tions such as occult bleeding appear, reticulocyte counts need not be done. Platelet counts, especially in thrombocytopenic patients, should be repeated occasionally. Although repeated liver and renal function tests showed no changes in our patients, further study of possible renal or hepatic damage is indicated. Patients should be weighed regularly and, if possible, caloric and fluid intake and fluid losses should be recorded in an attempt to determine the significance of weight losses in the treated patients. Although clinical studies are not well adapted to investigation of the mechanisms of urethane activity, biopsy material may be of great value in providing clues for such studies.

SUMMARY

The reports of urethane therapy of 90 patients with various types of malignant neoplastic diseases have been analyzed, and clinical, hematologic and histologic observations on 8 additional patients have been presented.

Favorable palliative effects have been obtained in cases of carcinomatosis, lymphoblastoma and leukemia. The most promising results have been reported in the treatment of androgen-independent carcinoma of the prostate and in the chronic leukemias. The results of treatment in chronic myelogenous leukemia are more favorable and less variable than in chronic lymphatic leukemia.

Decreases in the sizes of enlarged lymph nodes, spleen and liver, and a reversion toward a more normal peripheral blood picture have been observed in patients with leukemia.

Nausea and vomiting which occur in orally treated patients may be obviated by parenteral administration of the drug.

Toxic effects of importance include hypoplastic anemia, leukopenia and thrombocytopenia. The possibility of hepatocellular damage from urethane therapy needs further evaluation.

Urethane affects relatively undifferentiated leukocytes to a greater extent than the more differentiated forms. Erythroblastic tissue appears to be more resistant to urethane than is the case for leukogenic tissue. Megakaryocytes appear to be more resistant than either leukocytes or erythroblasts in the bone marrow.

Hematologic responsiveness or improvement is not always accompanied by clinical improvement in patients with chronic leukemia treated with urethane.

Weight loss, out of proportion to reduced caloric intake, may represent an important constitutional injury to patients receiving urethane.

Repeated blood and bone marrow studies, in addition to biopsy of tumors, lymph nodes, liver and spleen, whenever possible, may aid in determining the effects of treatment and mode of action of urethane.

Further investigation of urethane and its derivatives in the treatment of malignant neoplastic diseases in man is warranted.

REPORT OF CASES

Case 1

A 69 year old man was admitted with a history of weakness, weight loss and enlargement of lymph nodes, particularly in the right axilla. There was generalized enlargement of

lymph nodes which varied from 1 to 3 cm. in diameter. The edge of the liver was 4 cm. below the right costal margin and that of the spleen was 2 cm. below the left costal margin. Biopsy of a lymph node and study of the blood and marrow resulted in a diagnosis of lymphatic leukemic reticuloendotheliosis.

Oral urethane therapy was begun with an initial daily dose of 1.5 Gm. Within three days the dose was increased to 3.0 Gm. daily. A week after starting treatment the patient began to have anorexia. Two weeks after onset of treatment (40.5 Gm.) the axillary lymph node masses were definitely softer, smaller and more movable. Slight decreases in the size of the liver and spleen were also noted. The lymph node masses continued to regress, but the patient became progressively more cachectic. Repeated renal and liver function tests revealed no significant changes. The patient expired after fifty days of treatment and after having received a total of 106.5 Gm. of the drug. We were unable to obtain permission for autopsy.

Case 2

A 51 year old man known to have multiple myeloma was admitted because of fever, hemoptysis, cough and malaise. There was no bone pain or tenderness. There were Bence-Jones proteinuria, marked hyperglobulinemia and a reversed albumin-globulin ratio. The bone marrow contained myeloma cells. X-ray examination of the skull revealed areas of rarefaction.

Urethane therapy was started with an oral dose of 1 Gm. t.i.d. At no time was there anorexia, nausea or vomiting. The serum protein concentrations did not change and Bence-Jones proteinuria persisted. Serial x-ray studies showed no changes in the lesions of the skull. A definite drop in the leukocyte count was noted after 72 Gm. of urethane had been given. When the leukocyte count reached 2000 per cu.mm. urethane was discontinued. Since then there has been a gradual return of the leukocyte count to normal. Repeated renal and liver function tests revealed no changes during treatment.

Case 3

A 65 year old man with chronic myelogenous leukemia was admitted because of progressive weakness, dyspnea and edema of the ankles. The edge of the liver was 19 cm. below the right costal margin and that of the spleen was 3 cm. below the left costal margin. The lymph nodes were not enlarged. The initial blood study was characteristic of chronic myelogenous leukemia.

Urethane therapy was begun with doses of 1 Gm. t.i.d. After 25 Gm. of urethane were taken there was a marked fall in the total leukocyte count, with disappearance of immature myeloid cells. The patient never experienced anorexia, nausea or vomiting but during the period of urethane therapy lost 23 pounds of weight (initial weight 143 pounds). A great proportion of this weight loss may be attributed to the mobilization of edema fluid. After 49 Gm. of urethane had been given, the edge of the liver was 3 cm. below the costal margin and that of the spleen was 0.5 cm. below the left costal margin. No changes in renal or liver function tests were noted during treatment.

The patient was discharged with a normal leukocyte count. His physical status was much improved. He expired three weeks later of a "heart attack".

Case 4

A 61 year old man with chronic myelogenous leukemia was admitted because of progressive weakness. He had previously received x-ray treatment for leukemia; the last treatment had been given four months previously. The edge of the liver was at the level of the umbilicus and the edge of the spleen was 16 cm. below the left costal margin. The lymph nodes were not enlarged. The initial blood studies revealed a leukocyte count of 22,500 and a differential pattern typical of chronic myelogenous leukemia.

Oral urethane therapy was started with doses of 1 Gm. t.i.d. Before treatment was started the patient had mild anorexia without nausea or vomiting. After start of

treatment the anorexia increased and a continuous severe weight loss was observed throughout the period of therapy. It was felt that the weight loss was out of proportion to the reduction of food intake due to anorexia. After a total of 54 Gm. of urethane had been given, the leukocyte count dropped to 9750. Urethane was discontinued at this time, but it was resumed in the same dosage after ten days because of an increasing leukocyte count, discomfort from the enlarged spleen and continued weight loss. In an effort to alleviate the anorexia which was now associated with occasional vomiting, the total daily dose of urethane was given in the evening. Despite this measure the anorexia persisted. The leukocyte count continued to drop and after a total of 116 Gm. of the drug had been taken, it was 1900 per cu.mm. Therapy was discontinued, and supportive measures were used, the patient receiving transfusions and penicillin. However, there was a further continuous decline in the erythrocyte, leukocyte and platelet counts. During the subsequent sixty-nine days without further treatment there was marked subjective improvement, with gain in strength and appetite as well as a gradual increase in erythrocyte, leukocyte and platelet counts. Renal and liver function tests were unchanged.

Case 5

A 2 year old girl was admitted because of an upper respiratory infection, epistaxis and vomiting. There was a generalized enlargement of lymph nodes which ranged from 2 to 3 cm. in diameter. The initial blood study showed 17,000 leukocytes per cu.mm. with 47 per cent stem cells, anemia and thrombocytopenia. The hematologic diagnosis was sub-acute lymphatic leukemia in an acute or terminal stage. Soon after admission, epistaxis became more severe, and subconjunctival hemorrhages and tarry stools appeared. X-ray examination of the abdomen revealed an enlarged liver extending below the crest of the ilium, and probable enlargement of the spleen.

The patient received transfusions and penicillin in addition to urethane. The starting dose of the drug was 0.25 Gm. t.i.d. Vomiting was experienced twice, once after 3.75 Gm. of the drug had been given, and again after 21 Gm. There was no noticeable anorexia and no weight loss until approximately a week before death. After a total of 12 Gm. of urethane was taken, an increase in the sizes of lymph nodes occurred. At the same time there was an increase in the relative and absolute number of stem cells in the blood. The dosage of urethane was then increased to 0.5 Gm. t.i.d. but there was no effect on the progress of the disease. The liver and spleen remained about the same size but lymph nodes became slightly larger. Two days before death bleeding from mucous membranes began to occur. The patient expired after having received a total of 26.5 Gm. of urethane over a period of twenty-seven days. An autopsy was performed two hours after death.

Case 6

A 76 year old man was admitted because of the appearance of three rapidly growing tumors about the face and forehead and a fifteen year history of massive, progressive enlargement of masses in the cervical, axillary and inguinal regions. On one side of the nose there was an elevated, ulcerated lesion 1.5 cm. in diameter, with a pearly, circular border. Over the left temporal region there was a papillary mass 4 cm. in diameter with a completely ulcerated and hemorrhagic surface. Over the right temporal region there was an indurated fungating tumor 3 cm. in diameter. In the center there was a small superficial ulcer (Fig. 15). There were matted lymph nodes in both sides of the neck giving the patient a "chipmunk" appearance. Axillary, inguinal and supraclavicular nodes varied from 2 to 5 cm. in diameter. The edge of the liver was 4 cm. below the right costal margin and that of the spleen was at the level of the iliac crest. The tumors of the skin over the left temporal region and near the nose were removed surgically and the large tumor over the right temporal region was subjected to biopsy. All three proved to be squamous cell carcinomas. Biopsy of an axillary lymph node, and study of the blood and bone marrow established the diagnosis of chronic lymphatic leukemia. A specimen of liver tissue, obtained peritoneoscopically, revealed extensive leukemic infiltration.

Oral urethane therapy was started with 3 Gm. doses daily. Two weeks after onset of treatment the patient developed anorexia, but there was no nausea or vomiting. After the patient had received 71 Gm. of urethane, the leukocyte count, which was originally 126,500 per cu.mm., fell to less than one-third of its original value. Lymphoblasts were no longer present. During treatment the patient experienced slight dizziness at night on arising from bed. The dose of urethane was increased to 6 Gm. per day and this was well tolerated. After 191 Gm. had been administered, vomiting occurred after ingestion of the drug. This was obviated by reducing the dose to 3 Gm. daily. There was a 15 pound weight loss during treatment. As the leukocyte count was falling, the enlarged lymph nodes became softer, smaller and more movable. The liver was not noticeably decreased in size, but there was slight decrease in the size of the spleen. A second biopsy of the liver after 221 Gm. of urethane had been given revealed no change in the appearance or extent of leukemic infiltrations. The bone marrow also showed no decrease in the extent of lymphocytic replacement. After 107 Gm. of urethane, the remaining squamous cell carcinoma over the right temporal region developed a small area of necrosis at the site of incision for biopsy. Prior to this development, a prominent circular zone of hyperemia appeared in the skin around the edge of the mass. As treatment was continued, the necrosis extended until it ultimately involved the entire surface of the tumor. The superficial one-third to one-half of the tumor sloughed off. In the meantime, there was no lateral extension of the lesion. Successive biopsies revealed changes in the histologic appearance of the tumor, as described in the text above. Treatment was discontinued after 221 Gm. of urethane had been given because the patient refused further hospitalization. At the time of discharge the leukocyte count was 12,000 per cu.mm. The residual portions of the skin tumor were removed surgically before the patient left the hospital. Renal and liver function studies showed no changes during treatment.

After an additional eight days of observation in the outpatient department, during which period no further treatment was given, the leukocyte count rose to 29,450. The increase was due to an increase in the absolute number of small lymphocytes. The surgical wound continued to heal satisfactorily. Three weeks after discontinuing therapy the patient died of coronary thrombosis. Although an autopsy was performed elsewhere, we were unable to obtain tissues.

Case 7

A 55 year old man was admitted because of weight loss, dyspnea, cough and pain in the upper portion of the sternum. In the manubrium there was an elevated, firm, tender mass measuring 3 by 4 cm. There was a massive pleural effusion in the left side of the chest. X-ray examination revealed osteolytic lesions in the sternum, clavicles, ribs and sacrum, and massive left-sided pleural effusion which prevented accurate visualization of the lung. Biopsy of the sternal tumor revealed squamous cell carcinoma which was considered typical of metastatic lesions arising from bronchiogenic carcinoma. The clinical impression was carcinoma of the lung with skeletal metastases. Six grams per day of urethane were administered orally.

Four days after starting treatment the patient began to complain of slight anorexia. During the period of treatment signs of infection in the left pleural space were accompanied by the appearance of a septic type of fever, which continued for five weeks. Neutrophilic leukocytosis occurred during administration of urethane. The tumor in the sternum increased in size, and x-ray examination of the sacrum showed a definite increase in the size of the osteolytic lesions. There was a progressively unfavorable course and the patient expired after having received a total of 131 grams of urethane. During the entire course of treatment repeated renal and liver function tests gave negative results.

Case 8

A 54 year old woman with mycosis fungoides was admitted for a trial of urethane therapy. The patient had received several courses of x-ray therapy during the four years before ad-

mission. With each series of x-ray treatments the lesions in the skin faded in color and decreased in size. The pruritus diminished in severity but the response was transient. The last series of x-ray treatments had been given five months before admission. There were many circinate raised brownish red and erythematous lesions 2 to 5 cm. in diameter over various parts of the skin of the entire body with the exception of the palms and soles. Biopsy of a skin lesion revealed changes consistent with the diagnosis of mycosis fungoides. Blood and bone marrow studies indicated normal findings.

Oral urethane therapy was started with doses of 3 Gm. per day and increased to 6 Gm. per day. After 30 Gm. had been taken there was fading of the color of the lesions and a marked decrease in the severity of pruritus. The lesions of the face exhibited marked regression. The effect of urethane therapy was more rapid and greater than that previously obtained with x-ray therapy. There was no difficulty in taking the urethane until a total of 42 Gm. of the drug had been ingested. The patient then began to have nausea, and after several episodes of vomiting she refused further treatment. No other constitutional symptoms were noted. There was no change in the peripheral blood.

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THE DIAGNOSIS OF HISTOPLASMOSIS IN ULCERATIVE DISEASE OF THE MOUTH AND PHARYNX*

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Since the beginnings of pathology, emphasis has been placed on the relationship of the morphologic changes to the pathologic process. In the course of time certain patterns in tissue reactions have come to be associated with specific micro-organisms and pathologists have been willing to assume the responsibility of translating many of these changes into etiologic diagnoses. More comprehensive and detailed studies, however, have shown that, in certain instances, different organisms of widely varying biologic nature may incite similar histologic responses on the part of the host. Conversely, because infections vary with the virulence and the number of invading organisms and with the natural resistance of the host, a given type of infecting agent may, under different conditions, elicit varying degrees and types of histologic reaction, even though pure cultures of the organism may give rise to standard reactions in laboratory animals under controlled experimental conditions. With the development of chemotherapeutic and antibiotic agents it is becoming more and more important that the pathologist establish not only a histologic diagnosis but also the precise nature of the invading organism and whether it is susceptible to the recognized therapeutic agents and to the new ones being developed. Such determinations are in the best interests of the patient, the referring physician and the pathologist alike. Careful studies at the Mayo Clinic of surgical and necropsy tissues in the past have demonstrated certain limitations of histologic procedures when they are not supplemented by adequate bacteriologic investigation. Histoplasmosis is a disease which has been frequently misdiagnosed but which may readily be recognized and proved with a minimal amount of equipment and trained personnel, using the newer technics that are generally available.

In 1906, while looking for mucocutaneous leishmaniasis, Darling¹⁸ found an infection which he described as a new entity. In 1907 and 1908, he encountered two additional similar infections and suggested the designation "Histoplasma capsulata" for the organism.¹⁹ In 1926, Riley and Watson⁴⁹ reported the disease in a 52 year old woman who had not been outside of Minnesota for forty-two years, thus indicating that the infection was not likely to be entirely tropical in distribution. It is now recognized that the disease is essentially world-wide in distribution, although most of the cases have been reported from the United States.

In 1934, Hansmann and Schenken²⁷ reported on a patient with skin lesions of fifteen years' duration which, at autopsy, proved to be due to yeastlike organisms and which are now recognized as having been those of histoplasmosis. In 1934,

* Presented at the Twenty-Sixth Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 29, 1947. Received for publication, October 29, 1947.

Dodd and Tompkins²³ reported the first instance in which the diagnosis was made before death. Since that time many cases have been reported in which the diagnosis was made ante mortem.^{8,16,17,21,35,44,53,60} Increasing recognition of the disease as a clinical entity which is associated with a specific etiologic agent has become widespread, so that some of the cases recently reported in the literature actually pertain to patients who had died of the disease several years previously.⁶

In the reported cases the patients have ranged in age from 2 months to 77 years. All tissues of the body have been involved, but the extent of involvement of the individual organs has varied greatly from case to case. In some cases there has been general dissemination of the organism, but in others the infection appears to have been limited to the adrenals, lymph nodes or local ulcerated areas. With few exceptions the disease has been fatal in proved cases, but there is epidemiologic evidence (obtained by skin tests) that many patients recover and develop calcified pulmonary lesions simulating those of pulmonary tuberculosis. Humphrey³⁰ has emphasized the involvement of the spleen, liver, lymph nodes and bone marrow and has preferred to give the designation "reticulo-endothelial cytomycosis". In more recent cases, however, it has been shown that a generalized distribution of the organism in this system does not always occur and that local lesions may develop, such as suppurative arthritis and subcutaneous nodules or a wide variety of other local manifestations.

We have carefully reviewed the reports¹⁻⁶⁵ of 73 cases of histoplasmosis, not including 13 cited by Meloney³⁹ in which the clinical aspects are not recorded in detail. Several references in the literature have not been available to us and others have not contained sufficient clinical data to warrant interpretation. In the reports of 73 cases which we have reviewed, there have been such conditions as skin ulcers (face, neck, trunk and penis), cutaneous abscesses, subcutaneous nodules, generalized lymphadenopathy, purpura, perforated nasal septum, oral lesions (ulceration or induration, or both), peritonsillar abscess, laryngeal ulcers, mass in the epigastrium and ulcers of the rectum. The clinical signs and symptoms have included pain in the chest with productive cough, chills and fever, weight loss, nausea, vomiting, weakness, enlarged abdomen, painful defecation and diarrhea, with or without blood in the stool.

The clinical manifestations have been interpreted as syphilis (primary or tertiary), impetigo, breast abscesses, aleukemic leukemia, trench mouth, typhoid fever, Addison's disease, splenic anemia, suppurative arthritis, gallbladder disease, gastric ulcer and carcinoma. In a few cases the condition has been recognized clinically as probable histoplasmosis. The pathologic diagnoses in these cases, on biopsy, have been variously given as chronic adenitis, indeterminate granuloma, blastomycosis, lymphoblastoma, Hodgkin's disease, leishmaniasis, kala-azar, carcinoma, tuberculosis and histoplasmosis. In some of the cases the disease was associated with tuberculosis,⁴⁰ and in one case with cryptococcosis.⁴²

Of the reports of 73 cases which we have reviewed, in 37 the patients were 40 years of age or over, a period of life which might be called the "cancer age". Twenty-four of the 73 patients had oral lesions as part of the presenting complaint. Sixteen of these 24 patients with oral lesions (ulcers or induration) were

over 40 years of age. In the majority of reported cases the diagnosis has been made on histologic grounds alone. Because of the varied clinical manifestations and pathologic diagnoses coupled with our own experience, we should like to report 4 cases of ulcers of the oral cavity which illustrate the difficulties and uncertainties of the histologic diagnosis and emphasize the advisability of supplementing biopsy with bacteriologic study. For this purpose it is wise to arrange to hold separately an adequate representative portion of each tissue specimen from which reliable bacteriologic studies may be made if the histologic examination does not reveal a neoplastic process.

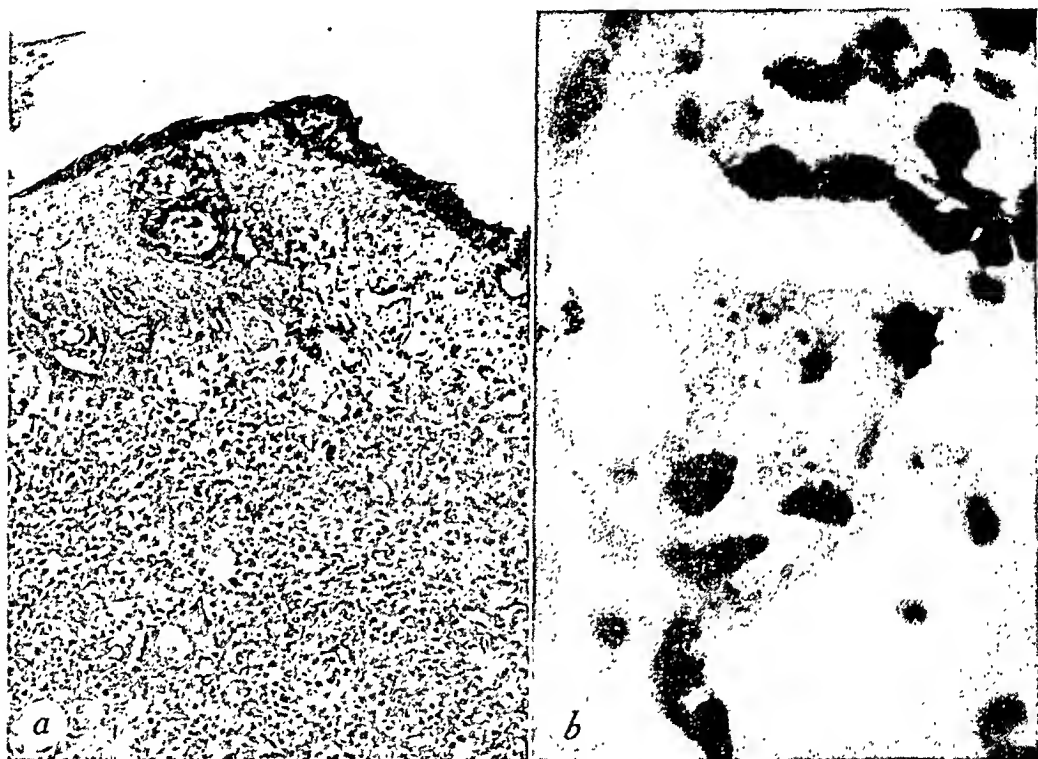


FIG. 1. (Case 1). Section from the left tonsillar pillar. a. Note the tendency toward epithelial hyperplasia and the numerous large macrophages immediately beneath the epithelium. The histologic interpretation is very easy with so many organisms in the tissue. $\times 150$. b. Macrophages shown adjacent to the epithelium in Fig. 1a. $\times 1200$.

REPORT OF CASES

Case 1

The patient was a 77 year old retired railroad engineer. For two months he had complained of soreness of the left side of the lower jaw. His dentist cauterized the lesion and filed down his plate, but the pain persisted. He had lost 30 pounds (13.6 Kg.) during the last four months. At the time of admission to the clinic there was an ulcerated area 1 cm. in diameter with heaped-up edges on the left anterior tonsillar pillar, a similar area over the mandible, one on the right tonsillar pillar and a smaller one on the epiglottis. Biopsy of the left pillar showed organisms with the morphologic appearance of *Histoplasma*, and a culture of the tissue removed for biopsy was positive for *Histoplasma capsulatum*. A similar organism was cultured from the urine. The histoplasmin skin test was negative. The

patient died nine months after onset of oral symptoms. The embalmed body showed persistence of the ulcer on the epiglottis and complete necrosis of both adrenals. Histoplasma was cultured from one of the adrenals.

Histologic examination. Biopsy of tissue from the pharynx showed a granulomatous lesion consisting chiefly of closely packed, large, pale, phagocytic endothelial cells associated with infiltration by leukocytes, chiefly neutrophils, and a few lymphocytes (Fig. 1a). The large endothelial cells were packed full of numerous intracytoplasmic organisms, 3 to 4 microns in diameter; these organisms were round or slightly ovoid, basophilic and had a clear halo-like capsule, typical of *Histoplasma* (Fig. 1b).

Case 2

The patient was a 37 year old merchant from Texas who had been well until 1940 when

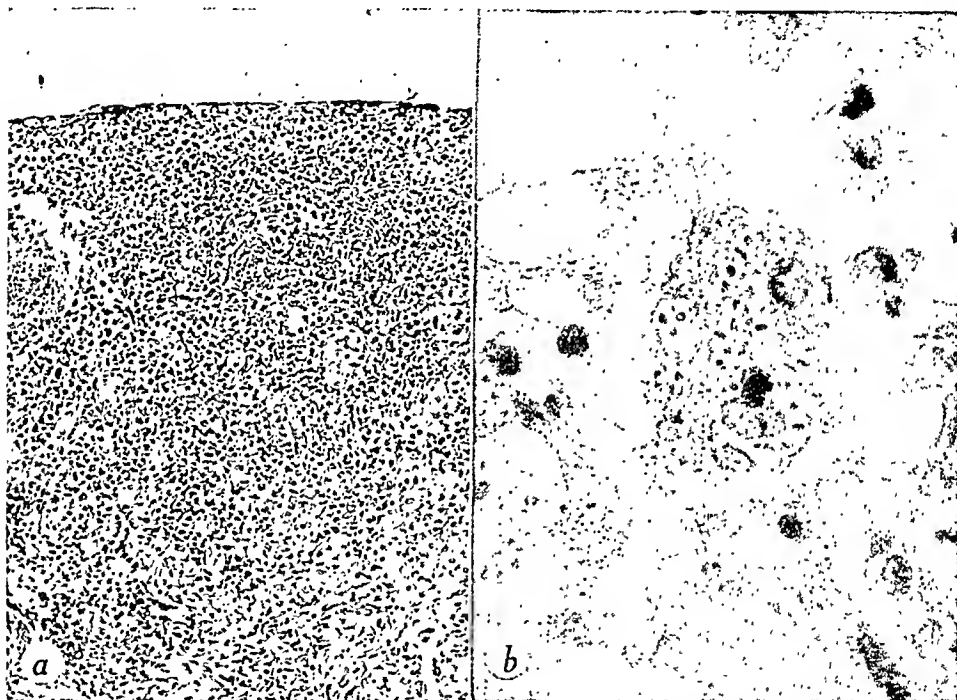


FIG. 2. (Case 2). a. Section of tissue from mouth shows numerous macrophages containing large numbers of organisms in the yeast form. $\times 155$. b. Portion of the tissue shown in Fig. 2a. $\times 1200$. Culture was positive for *Histoplasma capsulatum*.

he began losing weight and strength. In 1943, he was rejected by the draft board because of a pulmonary lesion. In 1945, he was ill for six or seven weeks with chills and fever of undetermined origin, for which he was treated with quinine. Recovery was gradual. In September 1946, a sore mouth developed and a diagnosis of scurvy and trench mouth was made. In December 1946, soreness of the mouth recurred, with an indeterminate lesion for which he was treated with streptomycin, sulfonamides and vitamins. This lesion persisted until he was admitted to the clinic in June 1947, with erosion of the corners of the mouth and the lower lip. The tip of the tongue contained several punched-out areas and the tongue could not be protruded because of pain. Biopsy of the tongue showed organisms the morphology of which was compatible with *Histoplasma* on direct examination. Cultures from the lesion and from the sputum were positive for *H. capsulatum*.

Histologic examination. Biopsy of tissue from the mouth showed an ulcerating, granulomatous lesion with ill-defined masses of large endothelial cells which were not arranged in

the form of "tubercles". Between and adjacent to these cells there was moderate infiltration with inflammatory cells, which in some areas consisted chiefly of polymorphonuclear leukocytes, in other areas chiefly of plasma cells. There was also an occasional multinucleated foreign body giant cell. Many of the large endothelial cells and the giant cells were packed with small, round or oval organisms with a dark central mass enclosed by a clear capsule (Fig. 2); these small organisms averaged about 4 microns in diameter, and had the morphologic appearance of *H. capsulatum*.

Case 3

The patient was a 48 year old man from Texas who had lived in Mexico from 1912 to 1915. He entered the clinic on June 12, 1940, with the complaint of weight loss and sore throat of two and one-half years' duration. In May 1938, he had become nervous, weak and subject to dizzy spells. In June 1938, all of his lower teeth had been removed. This procedure was followed by a severe sore throat for several weeks, which was treated with silver nitrate locally. He had begun losing weight and had run an afternoon fever but had had no cough, dyspnea, expectoration or hemoptysis. It had been customary for him to have an annual roentgenologic examination and the results had been negative for the past three years. The sore throat continued until April 1939, when a physician diagnosed a malignant disease and treated him with roentgen rays with some temporary relief. In August 1939, a recurrence of the ulcers of the mouth prompted re-examination and three biopsies were made; in each instance a nonspecific granuloma was reported. He experienced hoarseness, with difficulty in swallowing, and severe diarrhea which was associated with ulcers of the colon. Cultures made from the ulcers of the colon and from the lesions in the mouth showed "Monilia". He was treated with iodides until he became intolerant of them, and with this treatment did not improve. He was then treated with Monilia vaccine, which made his condition very much worse. The results of repeated tests for tubercle bacilli were negative. During the period of one and one-half years prior to admission to the clinic his weight fell from 217 to 109 pounds (98.4 to 49.4 Kg.) and he developed extreme weakness. One month before admission to the clinic he had had blood in the stools three times.

At the time of admission to the clinic the patient had enlarged submaxillary lymph nodes, the entire soft palate was gray, and the uvula was long and irregular and its mucosa contained many red nodules. There were numerous, gray, irregular nodules on the false vocal cords. Proctoscopic examination showed irregularly distributed superficial ulcers of the rectum and the sigmoid. Grossly, the ulcers were not typical of any specific infection. The examining physician believed they were too superficial for tuberculosis, too deep for bacillary dysentery and too large for amebic dysentery. Impression smears made from tissue removed for biopsy showed organisms which were interpreted as *Leishmania braziliensis*. On histologic examination the tissue from the rectum was interpreted as inflammatory. Tissue from the pharynx was interpreted as that of a granulomatous lesion, with intracellular organisms which probably were *Leishmania tropica*. The results of five separate examinations for acid-fast bacilli were negative. Impression smears from the lesion in the mouth, treated with Giemsa's stain, showed organisms which were interpreted as *L. braziliensis*. The results of cultures from the floor of the mouth were positive for *H. capsulatum*.

Histologic examination. Biopsy showed a diffuse distribution of numerous large foreign body giant cells, associated with small, poorly defined collections of large phagocytic cells (Fig. 3a) which were surrounded by a dense infiltration by leukocytes, consisting of few neutrophils, eosinophils and lymphocytes and large numbers of plasma cells, which extended through the tissue up to the covering epithelium. The epithelium was hyperplastic, showed proliferations which extended into the underlying tissues and, in places, simulated squamous cell carcinoma. On examination under high power, the cytoplasm of the giant cells and of many of the large phagocytic cells contained small, round or ovoid, dark-staining organisms having a clear capsule, and appearing morphologically typical of *H. capsulatum* (Fig. 3b).

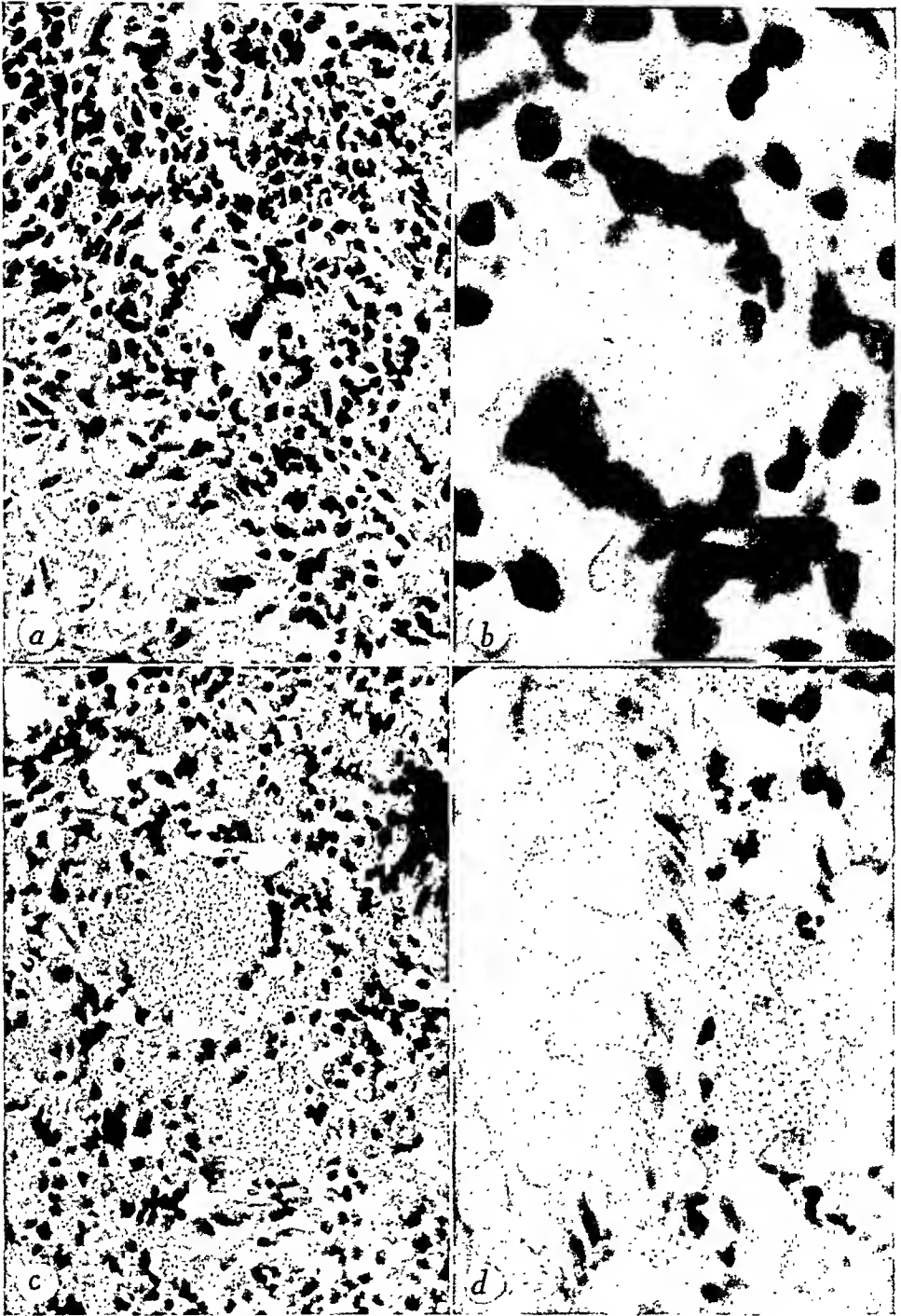


FIG. 3. (Case 3). a. Section of pharyngeal ulcer. Note the scarcity of macrophages containing *Histoplasma* as contrasted with the more recent lesion in the bowel shown in Figs. 3c and 3d. $\times 375$. b. Giant cell shown in Fig. 3a. $\times 1360$. c. Section of biopsy specimen of a recent rectal ulcer showing enormous numbers of organisms in the macrophages. $\times 375$. d. Parasite-laden macrophages adjacent to the glandular epithelium. $\times 680$.

Biopsy of tissue from the rectum showed chiefly large numbers of macrophages, associated with occasional giant cells, packed in the stroma between the mucosal glands (Fig. 3c). These cells were packed with enormous numbers of organisms (Fig. 3d) which were similar to those seen in the pharyngeal lesion.

Case 4

The patient was a 23 year old man who entered the clinic complaining of weakness, chronic fatigue, weight loss, low-grade fever and stomatitis of two and one-half years' duration. In May 1940, he had begun to lose weight and by September had lost 50 pounds (22.7 Kg.). During this time he was resting at home. In November, he began to have a

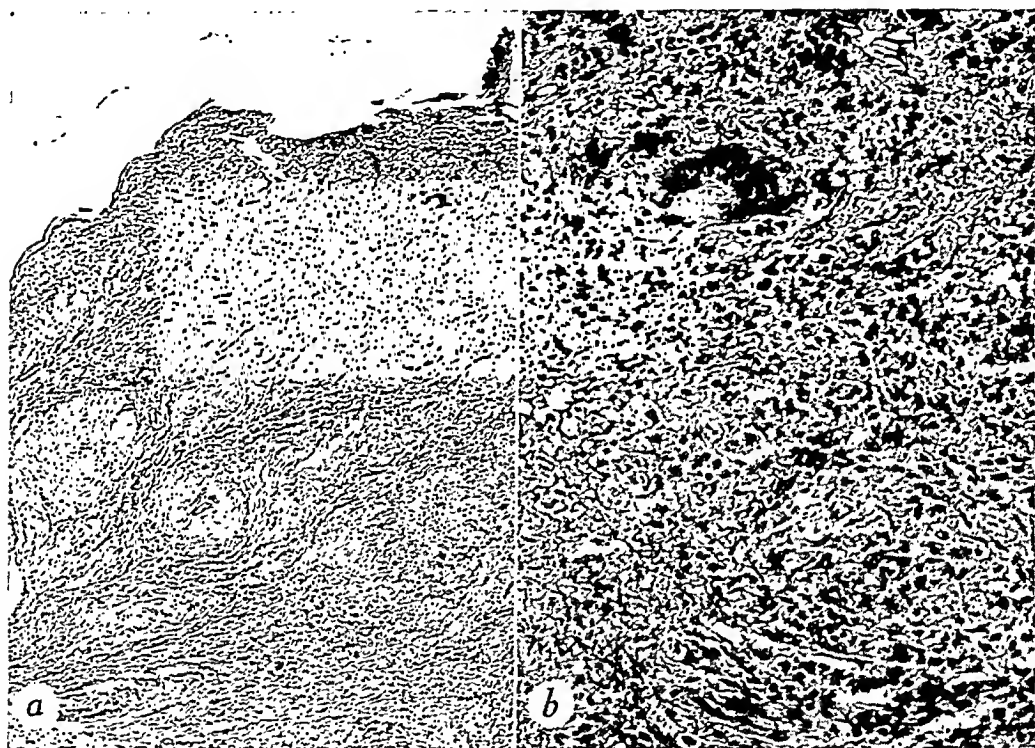


FIG. 4. (Case 4). a. Section of ulcer in the floor of the mouth showing granulomatous nature with giant cell formation. $\times 50$. b. Note absence of phagocytes containing organisms. No organisms suggestive of *Histoplasma capsulatum* could be found in the histologic sections. Cultures were positive for the organism. $\times 250$.

fever, up to 101 F. and profuse night sweats. By May 1941, he had improved and was able to return to work for a few days, but the weakness recurred and work had to be discontinued. In June 1941, he developed ulcers in the pharynx, bleeding from the gums and fever. The disease was diagnosed as trench mouth and he was treated for this condition, but the ulcers persisted. Roentgenograms showed clouding in the central portions of both lungs. He was sent to a sanatorium where his fever varied between 99 and 100 F. He was discharged in August 1941, because tubercle bacilli could not be demonstrated in his sputum.

During the next year the patient did no work. He rested and had plenty of food and fresh air, but he still felt fatigued, weak and short of breath on exertion and had occasional edema of the ankles. In August 1942, a blood test by his physician showed "spirochetal infection" for which he was given weekly intravenous injections for four months without improvement. During the rest of 1942 he had aching in the left hip and elbow joints, walked with a broad base and, at times, had a tendency to fall to the right side. Upon

admission to the clinic in January 1943, he was emaciated, had a sallow complexion, a pulse rate of 104 and a temperature of 99.8 F. There were granulomatous lesions on the soft palate near its posterior border, one on the hard palate, one in the left buccal fold opposite the bicuspid and a large ulcer involving almost the entire floor of the mouth. A fifth ulcerative lesion involved the tip of the epiglottis. Clinical impressions included blood dyscrasia, brucellosis, sarcoidosis, sarcoma, Hodgkin's disease, coccidioidomycosis or aspergillosis. On the basis of previous experience, one physician considered the lesions to be probably due to *Histoplasma*. A biopsy of the lesion from the floor of the mouth led to a diagnosis of tuberculosis. The results of guinea pig inoculation were negative for tuberculosis. Cultures of the material from the floor of the mouth were positive for *H. capsulatum*. *Histoplasma* were found in direct smears. The patient died in July 1944. Permission for autopsy was refused.

Histologic examination. Biopsy of a lesion of the mouth showed an inflammatory reaction. There were several nests of endothelial cells organized into well-formed "tubercles", with an occasional multinucleated giant cell, sometimes at the center, sometimes near the periphery of the tubercle (Fig. 4). At the base of an adjacent ulcerated area were unorganized masses of endothelial cells. Lymphocytic infiltration was almost absent; there was moderate infiltration with polymorphonuclear leukocytes, chiefly neutrophils, and near the ulcerated area there was, in addition, a rather diffuse infiltration with plasma cells.

COMMENT

In Case 1 the organisms were easily demonstrated by histologic examination and were interpreted as probably being *H. capsulatum*. In Cases 2 and 3 the original interpretations were more difficult because the clinical findings were much more confusing. The organisms, on biopsy, were visible, but were not properly interpreted until the diagnosis was established by culture. In Case 4, no organisms were visible on histologic examination so that the diagnosis had to be made entirely on the basis of culture alone.

The ulcers in Cases 1 and 2 were of relatively recent development and contained many organisms. In Case 3, the lesions in the oral cavity were of long duration and contained few visible organisms, but the rectal lesions were of short duration and contained many organisms which were easily recognized in the histologic sections. In Case 1 many organisms were in the oral lesions at the time of diagnosis. The ulcer on the epiglottis, however, which was not subjected to biopsy but which was presumed to have the same etiology as the pharyngeal ulcers, showed no recognizable organisms in the tissue sections at the time of autopsy. In this case the adrenals showed necrosis and no organisms were visible in the histologic preparations, but the cultures were positive for *H. capsulatum* even though the body had been embalmed six to eight hours. These findings may be interpreted as evidence that in some cases the organisms either tend to disappear from a lesion or become changed into some form which is not recognizable at the present time. The case of Parsons and Zarafonitis,⁴⁵ in which there were ulcers of the tongue that had remained healed for five years and the patient had remained apparently well, together with the epidemiologic evidence obtained by skin testing, supports the view that histoplasmosis may eventually be shown to be, like coccidioidomycosis, a common disease in which there is a fatal termination in only a small percentage of cases. This, however, is entirely speculative at present.

CONCLUSIONS

1. The clinical manifestations of histoplasmosis vary extensively and may appear as ulcerated or indurated lesions in the oral cavity simulating those of tuberculosis, malignant disease and leishmaniasis.

2. On biopsy the organisms may be present in the tissue in sufficient numbers to warrant a histologic diagnosis or the organisms may be so scarce as to render an etiologic evaluation impossible.

3. The organism, *Histoplasma capsulatum*, when present in tissue removed for biopsy may be easily isolated by inoculating the emulsified tissue onto blood agar containing 50 units each of penicillin and streptomycin per cubic centimeter of medium to inhibit bacterial growth. This is a suitable medium for other mycotic agents such as *Blastomyces dermatitidis*, *Coccidioides immitis*, *Cryptococcus hominis* and others.

4. Tissue for biopsy should be so handled that adequate material may be kept separately for suitable bacteriologic investigation if the histologic examination does not reveal a neoplastic process.

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THERAPY OF SEVERE ERYTHROBLASTOSIS FETALIS WITH REPEATED AND MASSIVE EXCHANGE TRANSFUSIONS*

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In previous papers,^{23, 24, 25, 26} we described a new method of treatment of erythroblastosis fetalis by exchange transfusion, and reported in detail 2 cases treated by this method. Up to September 1947, we have had experience with 25 cases, in 5 of which the infants died despite treatment. All of the infants who survived have recovered completely, at times with spectacular rapidity, unlike anything observed prior to the institution of this type of therapy. None of these patients has exhibited any evidence of permanent damage to brain or liver, and all have developed normally during periods of observation up to one year or longer. The purpose of this paper is to describe a modification in the handling of these infants, based on our experience with the first 25 cases, and to report successful results in 2 difficult cases.

In a typical case of erythroblastosis, the baby is born with Rh-positive red blood cells, coated with univalent Rh antibodies, and often also with free Rh antibody in its serum. If the cells break down by gradual lysis, a progressive hemolytic anemia results, which is readily combated by simple transfusions of Rh-negative blood, or in milder cases, even by transfusions of Rh-positive blood. Such infants, as a rule, recover without sequelae. If, in addition to or instead of undergoing hemolysis, the red blood cells should clump, either by conglutination or agglutination, organic damage will result and the baby will die, or if it survives, will develop permanent damage to the brain, liver and other organs. Obviously, such patients will not be helped by simple blood transfusions. Unfortunately, there is no way of determining with certainty in which cases lysis alone will occur and in which there will also be clumping. For optimal results, therefore, one must assume that clumping will occur in all cases and take steps to prevent this eventuality. Fortunately, clumping rarely, if ever, occurs while the fetus is *in utero*; the process seems to be initiated by the birth of the infant for reasons previously outlined in other papers.^{15, 16} (Caroli and Bessis² have found this to be true also of the analogous disease in mules.)

The prevention of clumping *in vivo* could theoretically be achieved by one of two methods. It is known that the type of blood which a person possesses has no effect on his or her health; whether one is Rh-positive or Rh-negative is of no consequence. It is also known that antibodies *per se* are not toxic, so that a strongly sensitized Rh-negative person is just as healthy as one without Rh antibodies. The simultaneous presence in the body of both Rh-positive red blood cells and Rh antibodies, however, may prove disastrous, as when a sensitized Rh-negative person is given a transfusion of Rh-positive blood, or when an Rh-positive fetus receives Rh antibodies passively from its sensitized mother

* Received for publication, October 16, 1947.

through the placenta. It is the latter situation which concerns us here. Two alternative courses of action suggest themselves, namely, either to remove the Rh antibodies from the infant's body, or to replace the Rh-positive blood cells by Rh-negative blood cells that cannot be clumped by the antibodies. The former is the basis of the therapy advocated by Darrow,⁴ Danis³ and others who treat these infants with transfusions of Rh-positive blood. This treatment is ineffectual, except in the mildest cases, for two reasons: (1) when antibodies are present in tissue fluids as well as in the plasma, as is usually true in the severer cases,²⁶ it is impossible to introduce enough red blood cells to absorb them completely; and (2) the addition of Rh-positive blood cells would have no effect on the infant's cells which are already coated with antibody and could still undergo clumping. Furthermore, the introduction of as large a volume of blood (250 cc.) into a newborn infant at a single transfusion without simultaneous withdrawal of blood, as has been suggested by some workers,^{1, 13} will almost invariably produce a dangerous plethora and polycythemia.¹⁰ The alternative then is to replace the Rh-positive blood cells with Rh-negative blood, a procedure that is both practicable and effective.

When performing an exchange transfusion, the main obstacle to obtaining a complete exchange lies in the necessity for carrying out the bleeding and the infusion simultaneously; as the transfusion proceeds one removes proportionately more of the blood that has been introduced and proportionately less of the baby's blood, so that a complete exchange is impossible. Until now, it has been our practice to introduce and remove about 500 cc. of blood, or twice the baby's blood volume, and thus attain an 87 per cent replacement.^{23, 26} As has already been pointed out, this has been sufficient to arrest the disease in the great majority of cases. We have, however, lost several patients, apparently because of intravascular clumping of the remaining 13 per cent of the infant's coated red blood cells. It had been hoped that lysis instead of clumping of these cells could be instituted by replacing some of the donor's plasma with saline solution to reduce the congenitally high content,²⁶ but this modification has not proved entirely effective and has been abandoned. The only alternative appears to be to resort to a more complete exchange transfusion, by using 1000 cc. of blood in the more severe cases, thus bringing about an exchange of approximately 98 per cent of the infant's red blood cells.

For the proper application of exchange transfusion therapy one must be prepared to carry out complete antenatal blood tests. These include: (1) complete Rh-Hr blood typing of the prospective mother, father and all living children, and sometimes of the prospective father's parents when necessary to determine his zygosity; and (2) periodic examination of the expectant mother's serum to determine its Rh antibody content.^{16, 21} The antibody titrations must be carried out by standardized methods if one is to be able to prognosticate at all accurately the severity of the illness in the fetus. We have found that the combination of the usual saline agglutination titration technic with the albumin-plasma agglutination technic²¹ is satisfactory, and that there is a good correlation between the results of the latter test and the severity of the manifestations in the infant. For

example, we¹⁸ have found that a titer above 50 units by the albumin-plasma conglutination technic usually means that the fetus will be stillborn if the pregnancy is allowed to go to term, and that the higher the titer the earlier the fetal death will take place; so that, with very high titers (above 150 units) fetal deaths may occur as early as the twenty-sixth week of gestation. It has been demonstrated by direct methods,^{17, 19, 22} that the univalent antibodies filter through the placenta as early as the sixth month and coat the fetal red blood cells. We have also found that a high titer of univalent antibodies (*e.g.*, 100 units) in the mother, acting for a short time, is more dangerous to the fetus than a low titer (*e.g.*, 10 units) persisting throughout the entire pregnancy. Univalent antibodies filter through the placenta readily and continue to pass through it until all of the fetal red blood cells are coated and enough additional free antibody has accumulated in the fetal plasma to make the titers equal on both sides of the placenta. Therefore, in known sensitized women, the antibody titers should be repeated at close intervals and the development of a high titer should be an indication for early termination of the pregnancy, but only with the understanding that the infant will be treated without delay by exchange transfusion. On the other hand, if the titer is low, premature delivery may be more hazardous to the infant than more prolonged exposure to the Rh antibody.

Thus, it is difficult to decide which pregnancies to allow to go to term and which to terminate early, and in the latter instances, how early to terminate them. The decision is particularly difficult if the husband is heterozygous since there is a 50 per cent chance that the fetus may be Rh-negative. In instances where the husband is heterozygous, our practice is to allow the pregnancy to go to term if the antibody titer fails to rise or declines as the pregnancy proceeds. On the other hand, we consider a *significant* rise in titer an indication for early termination of the pregnancy, for in our experience this has occurred only when the fetus was Rh-positive. Claims of the so-called nonspecific anamnestic reaction can be ascribed as a rule to the intrinsic inaccuracies of the titration methods and to too literal interpretation of results obtained on different dates.

REPORT OF CASES

Case 1

The patient was first seen by one of us (W.) on November 26, 1946, when she gave the following history.

Her first pregnancy terminated with the birth of an 8½ month male infant that weighed 5 pounds, 12 ounces. This child lived for only one-half hour and death was ascribed to narcotization with nembutal. A second pregnancy, in February 1941, yielded a male infant that weighed 9 pounds, 3 ounces. This child was neither jaundiced nor anemic during the neonatal period, developed normally, and is now living and well. Twins of unlike sex resulted from a third pregnancy in May, 1945. Both appeared to be normal at birth. The girl twin weighed 5 pounds 14 ounces, breathed spontaneously and had no injuries. Two days later jaundice was first noted and the liver and spleen were enlarged. The temperature rose to 102.4 F. The jaundice increased during the day and by evening was described as brown. On the following day respirations were labored, the legs were edematous, muscular twitchings and nystagmus were present. The infant died on the morning of the third day. Blood count at birth showed a hemoglobin concentration of more than 17 Gm. per

100 ml., and a red blood cell count of 5.61 million. The smear contained 842 normoblasts per 100 white blood cells. The infant died without exhibiting anemia. The male infant followed substantially the same clinical course. Jaundice developed on the second day of life, with hyperirritability, hepatosplenomegaly, followed by pulmonary hemorrhage and death. Hemoglobin concentration was maintained at more than 17 Gm., and as many as 328 normoblasts per 100 white blood cells were present in the blood smear.

According to the referring physician, the mother's blood was examined on July 9, 1945, seven weeks after the delivery of these children, by two laboratories and both reported blocking antibodies to be present.

At the time of our first contact with the patient, in November 1946, grouping and Rh-Hr tests gave the following results:

<i>Blood of</i>	<i>Group and Subgroup</i>	<i>M-N Type</i>	<i>Rh-Hr Type</i>
Husband	O	MN	Rh ₁ Rh ₁
Patient	A ₁	N	rh
Living Son	A ₁	N	Rh, rh

As expected, the patient was Rh-negative, the husband was Rh-positive, and the living child was also Rh-positive. Moreover, the husband was Hr-negative as indicated by the designation Rh₁Rh₁, and therefore *presumably* homozygous for the Rh factor, so that every child of this couple would be expected to be Rh-positive. Tests for the presence of Rh antibodies in the mother's serum at this time gave negative results in both the agglutination and the plasma-conglutination tests, so that the mother had evidently lost the Rh antibodies that were present following the birth of the erythroblastotic twins.

The patient returned on January 20, 1947, with the information that she was pregnant for the fourth time, and the expected date of confinement was October 7, 1947. Rh antibody tests now showed the following: agglutination test, negative; blocking test, positive, 1 unit; albumin-plasma conglutination test, positive, 48 units.

Thus, antibodies were again present in the mother's serum and since the husband was *presumably* homozygous for the Rh factor, another Rh-positive and therefore erythroblastotic infant seemed inevitable. When the patient requested a therapeutic abortion, this course was endorsed by us on the ground that her antibody titer was already high early in pregnancy, so that a stillbirth seemed likely, with only a slight chance that the fetus might prove viable and could be saved by exchange transfusion. Moreover, we have found that when the fetus dies *in utero*, toxemia and postpartum hemorrhage are not uncommon, so that the hazards for the mother are increased. After reconsideration, the mother decided to go through with the pregnancy.

Periodic antibody tests were done at intervals of approximately six weeks, with results that are summarized in Table 1. It is difficult to decide how much of the fluctuation in titers, recorded in Table 1, was due to technical limitations and how much was due to real variations in the degree of sensitization. In any event, the continuous presence of Rh antibodies of moderately high titer was an indication for early termination of the pregnancy. Accordingly, on September 4, 1947, when the pregnancy had progressed to thirty-five weeks, cesarean section was performed and a female infant weighing 7 pounds, 2 ounces was delivered. The infant exhibited no obvious stigmata of erythroblastosis, although the amniotic fluid was observed to be yellow, and there was a thin yellow streak on the umbilical cord. The liver and the spleen were not enlarged. The blood taken at birth was later reported as: hemoglobin concentration, 17 Gm. per 100 ml.; erythrocytes, 4.0 million per cu.mm.; leukocytes, 56,000 per cu.mm.; polymorphonuclears, 44; stab forms, 7; young forms, 9; myelocytes, 7; lymphocytes, 31; monocytes, 2; normoblasts, 9 per 100 white cells. The red blood cells were macrocytic.

An immediate exchange transfusion was carried out, 550 cc. of blood being injected and 475 cc. withdrawn during a period of sixty minutes.* The infant withstood the procedure

* For exchange transfusions we use only fresh citrated blood, not bank blood. Before the transfusion 500 cc. of blood is drawn into a bottle containing 60 cc. of citrate solution.

well and was sent to the nursery in good condition. Further studies revealed that as expected the infant was Rh-positive (group A, type MN, type Rh₁rh). The infant's red blood cells were coated with univalent Rh antibodies so that they failed to clump when mixed with anti-Rh₀ agglutinating serum. In addition, the infant's serum contained free Rh

TABLE 1
RESULTS OF ANTIBODY TESTS DURING PREGNANCY IN CASE 1

DATE OF TEST	ANTIBODY TITER* IN UNITS			
	Agglutination Technic	Blocking Technic	Plasma Conglutination	Albumin-Plasma Conglutination
Jan. 29	0	1	9	48
Mar. 6	0	1½	4	80
Apr. 10	0	1	4	26
May 27	0	1½	8	48
July 10	0	2½	2†	9†
Aug. 29	0	1½	—	32

* All titrations were done against cells of types Rh₁ and Rh₂, so that the values given represent averages of two or more titrations.

† These low titers were probably due to the poor quality of oxalated plasma used in the tests on that particular day, as shown by the lack of change in titer by the blocking technic.

TABLE 2
RESULTS OF BLOOD STUDIES ON PATIENT IN CASE 1

TIME OF TEST	AT BIRTH	12 HR.	24 HR.	48 HR.	4 DAYS	7 DAYS	13 DAYS
Hemoglobin, Gm. per 100 ml.....	17	17.5	15.5	16	15.5	14	14
Erythrocytes, million/cu. mm.....	—	5.89	5.04	5.63	5.02	4.5	4.66
Leukocytes/eu. mm.....	56,800	27,750	22,750	26,850	17,500	15,900	14,400
Nucleated red blood cells/100 leukocytes.....	9	6	6	3	0	1	1
Polymorphonuclears.....	44	42	43	60	63	46	31
Stab forms.....	7	23	13	14	7	15	5
Metamyelocytes.....	9	5	9	1	1	1	0
Myelocytes.....	7	2	2	0	0	0	0
Premyelocytes.....	0	2	2	2	0	0	0
Eosinophils.....	1	1	6	1	6	3	5
Lymphocytes.....	31	17	15	15	15	23	46
Monocytes.....	2	8	10	7	8	12	13
Icterus index.....	26	—	210	—	225	80	75

antibodies in a titer of 1½ units by the albumin-plasma conglutination technic. The icterus index of the cord serum was only 21 units.

By differential agglutination it was shown that approximately an 85 per cent replacement had been accomplished, that is, 15 per cent of the blood in the circulation was still Rh-positive at the end of the procedure. In view of the finding of complete coating of the fetal red blood cells, the high titer of antibodies in the maternal serum and the presence of free antibodies in the fetal serum, it was anticipated that the infant would show adverse symptoms despite the transfusion. On the following morning jaundice appeared and

deepened rapidly so that by evening the skin had assumed an orange tint. The liver and spleen were now palpably enlarged but there were no other abnormal findings. The baby took its formula well. A blood count taken at this time showed: hemoglobin concentration, 15.5 Gm. per 100 ml.; erythrocytes, 5.04 million; leukocytes, 22,750; nucleated red blood cells, 6 per 100 white cells; polymorphonuclears, 43; stab forms, 13; young forms, 19; lymphocytes, 15; monocytes, 10; eosinophils, 6. The icterus index had risen to 210 units.

It was believed that the only chance to save this infant lay in another exchange transfusion aimed at the removal of the remainder of the Rh-positive blood cells. Accordingly, the procedure was repeated using the radial artery on the opposite side for bleeding and recannulating the saphenous vein on the side used the day previously for the infusion. Again, 550 cc. of blood from a group A, type rh donor was injected and 480 cc. was removed. This procedure also was done without the appearance of any untoward symptoms and upon its completion, the condition of the baby seemed unchanged. However, 10 cc. of 10 per cent calcium gluconate was injected prophylactically. Except for some lethargy that was present for the next twenty-four hours the baby's progress was satisfactory. The jaundice at first deepened slightly and then began to fade. On the eleventh day of life the icterus index had fallen to 80 units, and by the twentieth day was only 25 units. The baby's condition was excellent on the day of discharge at three weeks of age. Differential agglutination tests done on the infant's blood after the second exchange transfusion showed that more than 99 per cent of the blood was now Rh-negative. The hematologic data are summarized in Table 2.

Case 2

This mother was referred to us by Dr. G. Brancato in the thirty-fifth week of her second pregnancy. Her first baby, a girl, born in 1942, weighed 10 pounds, was neither anemic nor jaundiced in the neonatal period and is living and well. Four days post partum the mother developed fever and then suffered a uterine hemorrhage for which she received one blood transfusion. It is not known whether the blood administered was Rh-positive or Rh-negative. The patient was referred for study because routine antenatal Rh tests done by Dr. Brancato had revealed her to be Rh-negative and sensitized to the Rh factor.

Grouping and Rh-Hr tests done on the father, mother and daughter showed the following:

<i>Blood of</i>	<i>Group</i>	<i>M-N Type</i>	<i>Rh-Hr Type</i>
Father	B	MN	Rh ₁ Rh ₁
Mother	B	MN	rh
Daughter	B	MN	Rh ₁ rh

Tests for the presence of Rh sensitization of the mother showed that, although no agglutinins could be detected in her serum, the blocking test was positive to 4 units and the albumin-plasma conglutination test was positive to 50 units. Inasmuch as the husband was presumably homozygous for the Rh factor, the expected infant would almost surely be Rh-positive. Therefore, in view of the high antibody titer of the mother's serum an erythroblastotic stillbirth could be expected if the pregnancy were to be allowed to go to term.

In our previous cases, no infant had survived if the mother had a titer as high as this, even if an exchange transfusion of 500 cc. was done immediately upon delivery. It was therefore decided that the infant should be delivered at once, despite the hazard that would be imposed by prematurity, and an exchange transfusion of 1000 cc. attempted. Since the mother and father both belonged to group B, the expected infant would belong to either group B or to group O. In either event blood from a group B donor would be compatible. Accordingly, on the day chosen for delivery, 1000 cc. of blood was prepared by bleeding two group B, type rh donors of 500 cc. each, while a cesarean section was performed by Dr. Bruce Harris.

The baby was a girl, weighing 4 pounds, 8 ounces at birth and exhibiting a waxy pallor. The vernix was yellow as was the amniotic fluid. An immediate exchange transfusion was started, and over a period of one and one-half hours 1000 cc. of blood was injected and 950 cc. withdrawn. During the course of the procedure, when about 600 cc. of blood had been

exchanged, the infant began to exhibit twitchings of the upper eyelids. Shortly thereafter, the skin became mottled and cyanotic, respirations became shallow and infrequent. Ten cc. of a 10 per cent solution of calcium gluconate was injected into the saphenous vein and the infant immediately breathed more normally and began to cry lustily. The untoward reaction was probably due to the large amount of citrate injected over such a short period of time. Aside from this, the procedure was without further incident, but an additional 10 cc. of the calcium gluconate solution was injected prophylactically at the end of the transfusion. The baby, in excellent condition, was then placed in an incubator.

A blood count taken at birth showed a hemoglobin concentration of 9.1 Gm. per 100 ml.; erythrocytes, 2.09 million per cu.mm.; and leukocytes, 33,900. After the first 500 cc. of blood had been injected the hemoglobin concentration was 10 Gm. and the red blood count 3.8 million. On the day following the procedure, the hemoglobin was 19 Gm. per 100 ml., the red blood count 6.2 million per cu.mm., and there were 143 nucleated red blood cells per 100 white blood cells.

Examination of the cord blood confirmed the prediction that the infant was Rh-positive. The complete classification of her blood was group O, type MN, type Rh₁rh. Actually, when the blood was typed, it failed to clump in anti-Rh₀ agglutinating serum, but this was due to complete coating of the infant's red blood cells by univalent Rh antibodies, as proved by the conglutination and anti-globulin technics. The cord serum had an icterus index of 50 units and contained free univalent antibodies, the titer by the albumin-plasma technic being 1½ units. The differential agglutination tests showed that the baby's blood after the transfusion was 100 per cent type rh.

Clinical jaundice developed on the second day of life, became pronounced on the third day and began to fade by the fourth day. A blood count on that day showed a hemoglobin concentration of 16.5 Gm.; erythrocytes, 6.3 million; leukocytes, 9100; polymorphonuclears, 56; stab forms, 5; myelocytes, 2; lymphocytes, 18; monocytes, 16; eosinophils, 3; and only 2 normoblasts per 100 white cells. The infant's prematurity prevented us from taking venous blood for studies of the icterus index. At no time did the infant appear to be ill, except on the evening of the third day of life when it exhibited a few tremors which were promptly terminated by the intravenous administration of calcium gluconate. The remainder of the course was uneventful, the jaundice disappearing within two weeks. When last seen, at the age of one month, the infant was making excellent progress, weighed 5 pounds, 1 ounce, and had a hemoglobin concentration of 15 Gm. per 100 ml. and a red blood cell count of 5.4 million per cu.mm.

COMMENT

While our technic has been described in previous papers,^{23, 24, 25} certain changes have been made based on our experience particularly with massive exchange transfusions. We are no longer concerned about any possible damage to the infant when the procedure is carried out rapidly, because the rates of inflow and egress of blood are so balanced that there is no danger of producing a dangerous plethora by speeding up the infusion, or of shock from too rapid withdrawal of blood. Since it is safe to speed up the procedure, a massive exchange transfusion involving 1000 cc. does not prove tiring either to the patient or to the operators. In order to guarantee a rapid flow of blood from the radial artery and to avoid any possibility of clotting, fifteen minutes are allowed for full heparinization before the arteriotomy is done. The radial artery is then freed, lifted up on a hemostat, and a flap cut by placing a fine scalpel at the center and cutting diagonally to one side; a rapid flow starts immediately and continues. Should the flow slow down, it can be started again by wiping the artery with gauze to remove any clot which may have formed. Since in this way an exchange transfusion of 1000 cc. can be completed within ninety minutes, the total amount of heparin required

is relatively small. From two to four doses of 0.2 cc. (200 units) each are ample,* the last dose being given at the middle of the procedure, so that there is little, if any, anticoagulant effect at the termination. Prophylactically, to counteract the probable hypocalcemia induced by the citrate in the infused blood, four doses of 5 cc. each of 10 per cent calcium gluconate should be given at even intervals during the procedure.

Of course, the exchange transfusion can be carried out in various ways, as has been pointed out by other workers.^{5, 9, 12, 14} The syringe method of Wallerstein,¹⁴ however, does not lend itself to the use of large amounts of blood and requires a high degree of technical skill. In experienced hands the umbilical catheter method of Diamond is satisfactory and some workers consider it simpler than the method described by us. It is, however, a blind procedure and would appear to be more tiring to the operators, since they must use syringes continually to aspirate as well as to inject blood. The method is not without some danger because at least two instances of death from air embolism have occurred.⁷ Technical failures, moreover, do occur when the umbilical vessels cannot be catheterized. In our own series of 30 exchange transfusions performed by using the radial artery for the withdrawal of blood and the saphenous vein for the infusion, we have not had a single technical failure, or any fatalities that can be ascribed to the procedure itself. The main objection to our procedure is that a greater amount of technical skill is required, especially to perform the arteriotomy. Once this is mastered the transfusion should progress without incident. The ligation of the radial artery, which is usually necessary at the end of the procedure, does no harm because adequate collateral circulation is present at the wrist.

As an additional precaution against reactions we check all donors used by us for the presence of Rh antibodies in their plasma. We also avoid the use of all donors who have a history of having received a blood transfusion or any blood injection. While the use of such donors would not be objectionable for adult Rh-negative recipients, their blood could conceivably aggravate the disease in the case of Rh-positive erythroblastotic infants (cf. Kelsall and Nicholas⁶).

In our series of 25 cases treated by exchange transfusions of only 500 cc. of blood, 5 died and 20 recovered completely. Of the 5 who died, 3 presented serologic and hematologic findings less severe than the 2 cases described in this paper. Accordingly, it seems reasonable to conclude that our percentage of recoveries in this series would have been higher if the more severely affected infants had been treated by massive exchange transfusion. If, as seems likely, the adoption of massive exchange in the more severe cases further improves our results, this would serve to refute the assertion that tissue damage in the disease is due to direct action of the Rh antibodies on tissue cells supposed to contain Rh antigen.† Chemical and serologic studies show that no significant changes re-

* The heparin used by us was kindly supplied by the Upjohn Company, Kalamazoo, Michigan.

† Incidentally, red blood cells coated with univalent antibody circulate and perform their functions normally, so long as hemolysis or clumping does not occur. In some infants studied by us in which coating of the erythrocytes was demonstrable at birth, the hemo-

sult from exchange transfusion in the concentration of various constituents of the blood plasma tested, such as plasma proteins, sodium, bilirubin and univalent Rh antibodies. The salutary effect of the procedure must therefore be ascribed entirely to the replacement of the coated Rh-positive red blood cells. Moreover, the argument that the "toxic substances" responsible for death result from the accumulation of the products of red blood cell hemolysis also seems to be incorrect, because in the most severely toxic cases, hemolysis occurs only in an insignificant degree, while on the other hand, the most severely anemic infants usually respond to simple blood transfusions. In the cases in which exchange transfusions of 500 cc. of blood were performed, leaving about 13 per cent of the infant's own blood to be hemolyzed, the yield of "toxic" materials would hardly be sufficient to account for the deaths in our series. This leaves the plausible explanation, which we have suggested previously,^{15, 16} that the toxic manifestations of icterus gravis are due to plugging of the blood vessels by clumping of red blood cells, thus compromising the circulation to vital organs.

When prognosticating the results of a pregnancy on the basis of antenatal antibody titrations, the presence of Rh sensitization should not blind one to the possibilities of other complications which may arise. For example, we have encountered a number of women with latent diabetes who have given obstetric histories indistinguishable from those in erythroblastosis. Miller *et al.*⁸ have emphasized the similarity between the pathologic findings obtained in stillborn fetuses of such mothers and those with proved erythroblastotic fetuses. Glucose tolerance tests are therefore indicated in cases where the severity of the disease is out of proportion to the serologic findings in an Rh-negative mother.

Without detailed serologic studies, in particular antibody titrations, it is not possible to evaluate the severity of the disease, and good results which are due to the mildness of the disease and spontaneous recovery may be ascribed incorrectly to the treatment.^{1, 7a, 13} The practice of giving "prophylactic" transfusions to babies of all Rh-negative women, including those without Rh antibodies, must be condemned, because such babies are not erythroblastotic and require no treatment. If the results of treatment by two different methods are to be compared, it must be shown that the cases in the two series are comparable in severity by complete serologic as well as clinical and hematologic data. Thus, the two cases presented in this paper furnish conclusive evidence of the value of the treatment used by us, because previous experience has proved that stillbirths were inevitable when the pregnancies were permitted to go to term, or that the infants died when the pregnancies were interrupted and the newborn were treated in the usual manner by simple blood transfusions or a small exchange transfusion.

SUMMARY

In two cases where antenatal Rh tests indicated that stillbirths were inevitable if the pregnancies were allowed to go to term, the pregnancies were terminated prematurely by cesarean section and the infants treated by exchange transfusion.

globin concentration did not fall to a level requiring transfusion for periods as long as three weeks.

In the first case, an exchange transfusion of 500 cc. of blood was given. When the infant exhibited marked progress of the disease despite this treatment, the exchange transfusion was repeated on the following day, after which the progress of the disease was arrested and recovery followed. In the second case, an infant weighing only $4\frac{1}{2}$ pounds at birth, received a massive exchange transfusion in which 1000 cc. of blood was injected and 950 cc. removed. This infant became jaundiced but showed no signs of toxicity and the jaundice cleared within a fortnight.

The gratifying results obtained in these two difficult cases most likely can be ascribed to the more complete replacement by Rh-negative cells of the infants' Rh-positive cells which were coated by antibody. Certain simplifications in our technic of exchange transfusion are described which speed up the procedure and make it less tiring to both the patient and the operators. The relative merits of the authors' method and the methods used by other workers are discussed.

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TRAUMATIC SACCULAR ANEURYSM OF THE THORACIC AORTA*

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The role of external trauma in the pathogenesis of disease of internal organs is a problem of constant importance in civilian practice and in military medicine. Trauma that produces an external wound with continuity to internal structures is the usual occurrence, and the relation between the trauma and the disease is then obvious. This is not so in those instances in which there is no external evidence of the trauma and yet injury to underlying viscera occurs.

The cardiovascular system may be damaged by external trauma and subsequently evidences of diseases may develop. Involvement of the heart in such a manner is not too rare, and there have been reported¹³ many instances of acute and subacute bacterial endocarditis, incompetence of valves and irregularities of cardiac rhythm in the pathogenesis of which trauma appears to have been a major factor. Less frequently has involvement of the aorta been seen, especially when no prior lesion existed. The smaller size, tougher wall, more protected position and tendency to slip out of the way when struck have been suggested³ as reasons for the less frequent damage of the aorta. Complete rupture may result, however, either from direct violence or from overstretching and overstrain. Traumatic aggravation of pre-existing disease, especially syphilis, may occur.

Development of an aneurysm in a previously normal aorta is the least frequent of the effects of trauma. The aneurysm is secondary to an incomplete tear of the wall; the occurrence of such tears has been discussed by Peery.⁹ In none of the 6 cases he reported had a true aneurysm developed; the healed lesion was more comparable to a healed erosion or ulcer. Development of a dissecting aortic aneurysm secondary to trauma was reported by Aschoff.² The lesion occurred in a 40 year old soldier who was wounded by a "tangential" shot at the level of the fourth rib. Death occurred suddenly four months later. Autopsy revealed a 4 cm. broad rent in the media of the aorta immediately above the aortic valve, with aneurysm of the intima. There was no evidence of other arterial disease. Leonard's⁷ patient was injured in an automobile accident sixteen days before death. The patient was pinned between the steering wheel and the back of the driver's seat. At autopsy a transverse fracture of the manubrium was found; posterior to this a dissecting aneurysm of the thoracic aorta had ruptured into the left pleural cavity. In this patient, aged 60 years, the aorta showed "atheromatous changes without calcification". While these lesions may have been important in the development of the aneurysm, the causal importance of the trauma appeared to be beyond doubt. A dissecting aneurysm of probable, but not definitely proved, traumatic origin was described by Samson.¹²

* Received for publication, October 20, 1947.

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Instances of traumatic saccular aneurysm of the abdominal aorta have been presented by Pick,¹⁰ and by Risen and Dickens.¹¹ Development of saccular aneurysm of the thoracic aorta secondary to trauma and apparently directly related to the trauma is most unusual. Heller, in 1903,⁴ described a lesion of this type. A man of 37 years fell while carrying a heavy weight, with the mass falling on his chest. He died eleven months later with signs of aortic insufficiency and autopsy revealed incompetence of one of the commissures of the aortic valve proximal to a saccular aneurysm of the first portion of the ascending aorta. There was no evidence of syphilis, but plaques of "endarteritis" were found in the arch of the aorta. Kaufmann⁵ saw a "saccular aneurysm as large as a walnut in the thoracic part of the otherwise perfectly healthy aorta of a sturdy young man. It had developed some months after a severe fall."

Recently there has been observed in the necropsy service of the Department of Pathology of the University of Michigan an instance of saccular aneurysm of the aorta which appeared to be of traumatic origin. In addition, examination of the records of the department disclosed that among persons dying shortly after severe physical trauma, there were two instances in which incomplete tearing of the aorta occurred. These two cases illustrate a first stage in the development of traumatic aneurysm.

REPORT OF CASES

Case 1

An 18 year old girl (A-247-AW) was admitted to the University Hospital with complaints of pain in the right breast and fever. Her symptoms had followed injuries received in an automobile accident five months previously. In the accident she suffered injury to her right breast, right hand and wrist, and the back of her head. The right breast had been swollen for about two weeks and had been intermittently painful since that time, with the pain chiefly in its medial portion. Suboccipital headaches occurred for one month following the accident.

Two months following the accident the patient developed an upper respiratory infection, and subsequently fever and chest pain. The pain was believed not to be of pleural origin. She did not have cough, sputum or chills. A diagnosis of pneumonia was made and she was placed at bed rest. Convalescence was characterized by marked weakness. Two months later, and four months after the accident, she again developed an upper respiratory infection, became very weak and experienced a shaking chill followed by fever. She also noted severe pain in the chest which was related to breathing. During convalescence from this illness she was first told that she had "heart trouble". Temperature was elevated daily to 102 F. There was one episode of paroxysmal dyspnea. On several occasions while lying on her left side she experienced precordial pain which was relieved by change in position. The patient was weak and anorectic and had lost 30 pounds in four months.

At admission to the hospital the temperature was 99.8 F. The veins in the neck showed prominent pulsations. No abnormalities could be palpated in the right breast; slight tenderness was noted on pressure over its medial aspect. The cardiac border was 10 cm. to the left of the midsternal line in the fifth interspace, and 3 cm. to the right of the midsternal line in the fourth interspace. A thrill was palpable over both the apical and aortic areas. There was tachycardia (110 per minute); rhythm was regular. A loud harsh systolic murmur was heard over the aortic area, and there was a questionable aortic diastolic murmur. Loud systolic and diastolic murmurs were audible over the apical area. Lungs were clear to auscultation. There was no peripheral edema.

A Kahn test of the blood was negative. There were no significant abnormal findings by urinalysis. Studies of the blood revealed 59 per cent hemoglobin, and 2,900,000 erythrocytes and 13,000 leukocytes per cu. mm., with a differential count of 62 per cent polymorphonuclear leukocytes, 30 per cent lymphocytes, 5 per cent monocytes, and 3 per cent eosinophils. Roentgenologic examination of the chest showed marked cardiac enlargement, chiefly to the right. The superior retrocardiac space was reduced. An electrocardiogram disclosed no abnormality. The P-R interval was 0.20; the QRS, 0.08 second. One blood culture showed no growth, but two others showed delayed growth of "a gram-negative diplococcus having the cultural and morphologic characteristics of the gonococcus. Antigonococcal serum was not available to prove the identity serologically."*

During hospitalization the patient had irregular elevations of temperature to 104 F. On the third hospital day she was started on large doses of acetylsalicylic acid with sodium bicarbonate. Temperature remained normal after the fourth hospital day. There was some nausea induced by the medication but no tinnitus was reported. On the morning of the ninth hospital day dulling of sensorium, slurring of speech and hyperventilation were noted. Salicylates were discontinued and efforts made to increase fluid intake. Vaginal bleeding occurred but no other hemorrhagic phenomena were noted. That evening physical signs of pulmonary congestion appeared. The patient became disoriented and was intermittently comatose. Nonpitting edema of the extremities was present. Urine output was scanty. Rapid labored respirations were not relieved by oxygen therapy and continued until respiratory depression occurred on the eleventh hospital day and the patient died.

Necropsy was performed by Dr. Marjorie M. Everett. There were no petechial hemorrhages in the skin or oral and ocular mucous membranes. The heart was moderately enlarged, weighing 440 Gm. There was fibrinous exudate on the epicardial surface. The mitral and aortic valves revealed a subacute stenosing valvulitis of rheumatic type. On the posterior surface of the aorta, about 1.5 cm. above the aortic cusps, there was a defect with diameters of 2.5 and 3 cm. To the right this defect opened into a sacular projection measuring 4 x 5 cm. and filled with mural thrombus. The major portion of the sac lay on top of the heart posterior to the aortic and pulmonary arteries. On the opposite side of the aorta just above a cusp of the aortic valve was a smaller defect about 8 mm. in diameter. Its edges were rolled and the floor was composed of opaque white tissue. The cavity of this sac was 1 cm. in greatest depth, and extended underneath the aortic wall distally for about 3 mm. Adjacent to the larger defect was a slightly depressed area in the aorta measuring about 1 cm. in greatest diameter. This was also located immediately above the aortic cusps. The gross appearance of these lesions is shown in Figure 1.

Microscopic examination of the heart revealed active chronic mitral and aortic valvulitis, subacute verrucous mitral endocarditis, diffuse active chronic purulent interstitial myocarditis and subacute fibrinous pericarditis. No Aschoff bodies were seen. Microscopic examination of the small aneurysm revealed a moderate fibrosis of the intima of the aorta at the proximal edge of the defect, with less marked fibrosis at the distal edge. The walls and floor of the sac were composed of a layer of moderately cellular fibrous tissue in which a few lymphocytes were included. The aneurysmal bulge extended under the adventitia distally, as seen in the gross examination. Connective and adipose tissues of the

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FIG. 1. Case 1. The two defects in the ascending aorta are shown, the smaller one to the right. Thrombotic material partly fills the interior of the larger saccular aneurysm.

FIG. 2. Case 1. Section through the larger aortic aneurysm. The elastic fibers are interrupted at the margins. Verhoeff's stain for elastic tissue.

adventitia were infiltrated by lymphocytes, plasma cells and large mononuclear cells; there were also phagocytic cells containing hemosiderin, indicating old hemorrhage. In some areas cellular infiltration and vascular proliferation formed small foci of granulation tissue in which nerve trunks were involved. In sections stained with the Verhoeff stain for elastic tissue, elastic fibers of the aortic media were seen to be interrupted and frayed at the edges of the aneurysm.

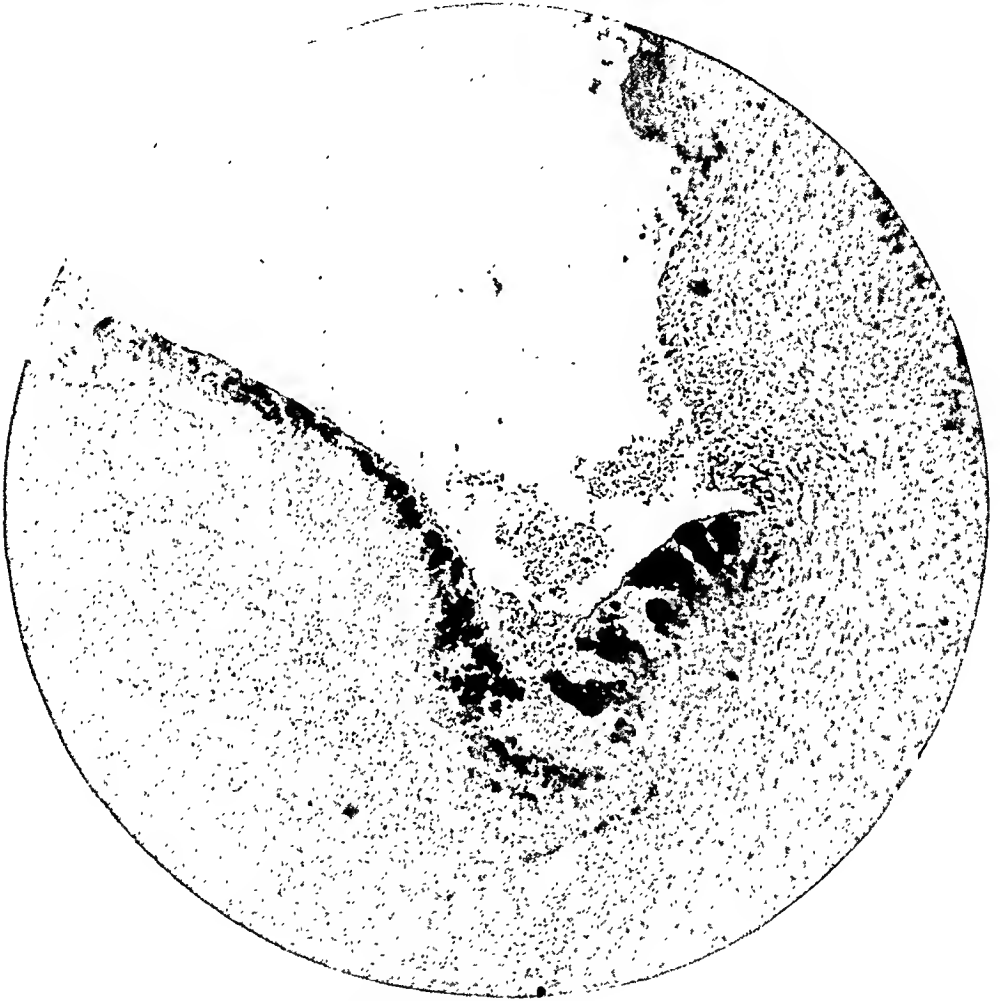


FIG. 3. Case 1. Section through a deeper portion of the aneurysm showing masses of bacteria and fibrin of the thrombus.

In the walls and base of the sac were scattered fragments of elastic tissue. At the distal edge of the sac the interrupted ends of elastic fibers were tangled together into a dense rounded mass suggesting retraction of broken fibers. Secondary inflammation was not present in this aneurysm.

The edges of the larger aneurysm showed a picture similar to that seen in the smaller sac, both in sections stained with hematoxylin and eosin and in those stained for elastic tissue. An organizing thrombus was attached to a portion of the wall of the sac. The interrupted elastic fibers again formed a tangled mass at the edge of the lesion (Fig. 2), and there were scattered individual

elastic fibers and small masses of fibers in the walls and floor. In addition, an acute purulent exudate was seen both in a portion of the cavity of the sac (Fig. 3) and throughout the fibrous tissue of the wall. The connective tissue was necrotic and showed focal calcification and extensive polymorphonuclear leukocytic infiltration (Fig. 4). Numerous bacterial colonies were present in the necrotic tissue and in the exudate.

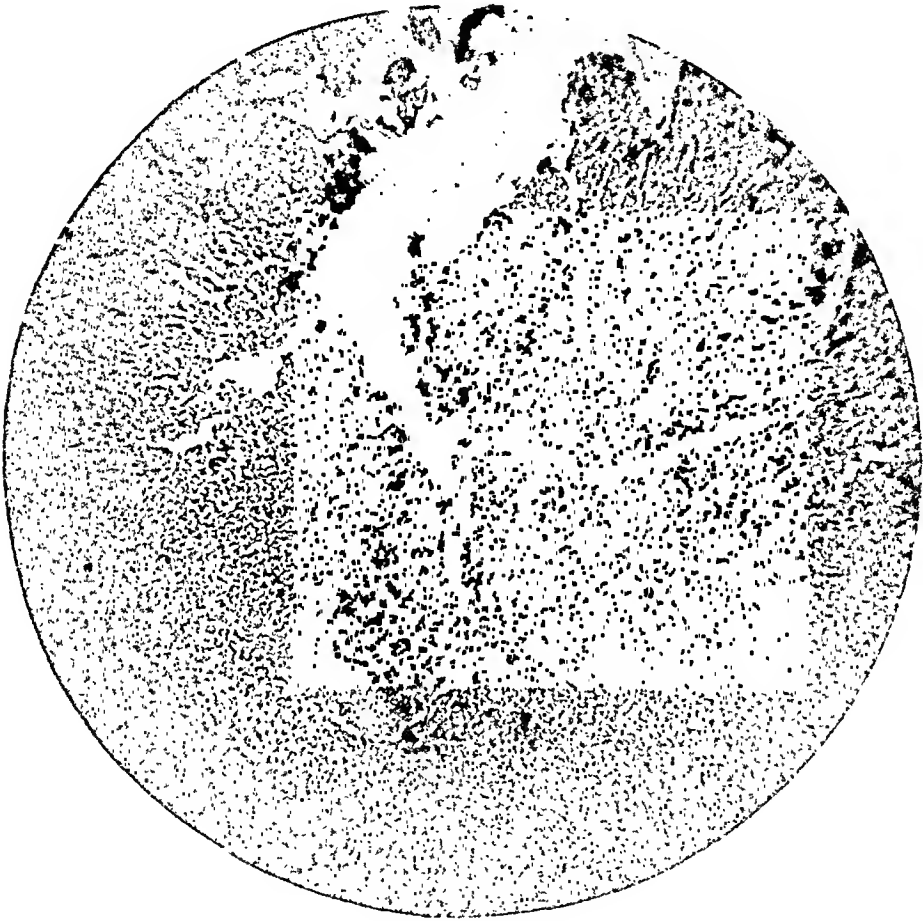


FIG. 4. Case 1. Marked purulent inflammation in another deep portion of the aneurysm.

A section through the slightly depressed area adjacent to the larger aneurysm showed no elastic tissue in the wall. Small foci of granulation tissue, including phagocytes with hemosiderin, were present. This lesion was probably a smaller area of rupture, not large enough to be considered an aneurysm.

Pathologic diagnosis. Subacute bacterial endoarteritis superimposed upon a saccular traumatic aneurysm of the first portion of the ascending aorta; bacteremia (gonococcal?); second smaller saccular traumatic aneurysm of ascending aorta, and small healed incomplete rupture; organizing fibrinous pericarditis and verrucous mitral valvulitis superimposed upon older active chronic processes;

active chronic aortic valvulitis; moderate stenosis of left cardiac valves; diffuse active chronic purulent interstitial myocarditis; mycotic embolus in cerebral arteriole with localized encephalomalacia; mycotic thrombus in meningeal vein; acute purulent leptomeningitis and pachymeningitis on older fibrous meningitis; acute purulent pelvic peritonitis; active chronic purulent cervicitis; bilateral active chronic salpingitis; "salicylate intoxication".

Case 2

A 66 year old man (A-4-AO) died three hours following injury in a collision of two automobiles. Heart sounds during this period had been very faint and the pulse was weak. These findings were believed related to the state of shock. Blood pressure was 80/60 mm. of Hg at the time of admission, and rose to 120/60 shortly before death.

Autopsy, performed by Dr. J. C. Bugher, revealed multiple fractures of the bones of the face, marked pulmonary fat embolism and moderate cerebral fat embolism. The pericardial sac was tense and bulging and contained about 100 cc. of dark nonclotted blood. In the ascending aorta, 5 cm. above the posterior aortic valve, was a transverse rent 4 cm. in length. This did not extend through the pericardium, but permitted escape of blood into the pericardial sac and effusion upward along the arch of the aorta.

Case 3

A 20 year old man (A-256-AE) died five days following collision between a train and the automobile in which he was a passenger. He did not regain consciousness following the accident. The only evidences of cardiovascular damage noted clinically were gradual decrease of the blood pressure and a weak pulse which were believed related to the condition of shock.

Autopsy, performed by Dr. C. H. Fortune, revealed multiple small petechiae throughout the cerebrum, moderate pulmonary fat embolism and terminal acute purulent bronchitis and hypostatic lobular pneumonia. In the first portion of the descending thoracic aorta, just distal to the arch, was a linear oblique laceration, 3 cm. in length (Fig. 5). The edges of this laceration were slightly rolled and gaping. The laceration extended only through the inner portion of the aorta, forming a cavity which extended about 1 cm. in either direction from the borders of the laceration. The cavity and its opening were partly filled by a firm clot.

COMMENT

Traumatic lesions of the thoracic aorta, whether incomplete tears, aneurysms or complete ruptures, usually occur in the first portion of the ascending aorta just above the aortic valve. Several factors which may explain this localization have been suggested. Peery⁹ described the pulling action on the aortic wall by the commissures of the aortic valve as the valve is forced shut in diastole, and considered this an important mechanism in causing tears. The fact that tears in this area are commonly transverse to the axis of the commissures was believed to support this hypothesis. The first portion of the aorta is a compara-

tively fixed area due to the fibrous union between the aorta and the pulmonary artery. In the application of sudden traumatic force this fixed point could not as successfully evade the blow as could a more movable area. The points of



FIG. 5. Case 3. Linear oblique laceration in first portion of descending thoracic aorta. A blood clot fills the tear.

junction of a fixed and a movable segment are also especially susceptible to strain. Another frequent site of rupture of the thoracic aorta is just distal to the entrance of the ductus botalli and to the exit of the main branches from

the arch. This is also a comparatively fixed point and particularly susceptible to sudden strain.⁶ Congenital weakness of the first portion of the ascending aorta has been described by Abbott,¹ but this is usually found in patients with other vascular abnormalities such as coarctation or bicuspid aortic valve. A sudden rapid rise in blood pressure at the moment of trauma is likewise important; this probably acts in conjunction with the other factors. Of these possible causes, the anatomic fixation of the vessel is considered the most important in localizing the lesions. Experimental work by Oppenheim⁸ has corroborated the vulnerability to excessive force of the first portion of the aorta.

The age of the patient in Case 1, 18 years, is an additional basis for believing her aneurysms to be of traumatic origin, since aneurysms developing spontaneously or on the basis of other vascular disease are uncommon in young persons.

The development of subacute bacterial endocarditis in the cavity and wall of the aneurysm in Case 1 is also noteworthy. Subacute endocarditis is usually found on congenitally abnormal valves or at sites altered by previous disease. Traumatic lesions of valves have been described¹³ as the site of endocarditis although here the true sequence of trauma and inflammation may be more difficult to determine. The occurrence of endocarditis, however, is rare, and it is highly improbable that this disease was existent in this case at the time of the accident; the existence of the smaller aneurysm without inflammation affords further demonstration of the primary nature of the traumatic lesions. The infection developed in a damaged area of the aortic wall and thus is comparable to development of endocarditis upon damaged cardiac valves. It is of interest that superimposed bacterial infection is not seen in syphilitic aneurysms, nor is there development of endocarditis upon valves damaged by syphilis.

The isolation of gram-negative cocci, apparently gonococci, from two blood cultures indicates a gonococcal bacteremia, and it is probable that the endocarditis was also gonococcal. Although cultures of the contents of the aneurysmal cavity were not made, the bacterial colonies seen microscopically were gram-negative in sections.

Interpretation of the cardiac physical findings in Case 1 is difficult because of the coexistent mitral and aortic valvulitis and pancarditis. These lesions could account for the thrill and the systolic and diastolic murmurs. In general, the presence of heart murmurs is of major importance in the diagnosis of incomplete rupture of the aorta or of a small saccular aneurysm. Such murmurs are chiefly systolic and are best heard in the aortic area. They are believed to be caused by the flow of blood over the edges of the tear. If the tear has caused insufficiency of the aortic valve, a diastolic murmur may be present. Electrocardiography should not be expected to aid in diagnosis. Left axis deviation might be present if hypertrophy of the left ventricle has occurred, but this would be nonspecific. Only in large and favorably placed aneurysms would roentgenologic demonstration be possible; an enlarged left atrium due, as in this case, to stenosis of the mitral valve, would increase the difficulty.

SUMMARY

A saccular aneurysm of the thoracic aorta following trauma is described. The lesion occurred in an 18 year old girl who died six months after an automobile accident in which there was injury to the chest. Subacute bacterial endoaortitis developed in the aneurysm. Incomplete tears of the aorta following trauma are described in two other patients. Aneurysm, either saccular or dissecting, endoaortitis, or aortic insufficiency if the tear is close to the valve, may be considered compensable lesions in properly selected cases.

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TUBERCULOMA OF THE MYOCARDIUM IN A PATIENT WITH TUBERCULOUS MENINGITIS TREATED WITH STREPTOMYCIN*

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Tuberculosis of the myocardium is infrequent, particularly when unassociated with tuberculous pericarditis. In an extensive review of the literature, Horn and Saphir⁵ found 19 cases of tuberculosis of the myocardium in 7683 autopsies of patients who died of generalized tuberculosis. This is an incidence of 0.24 per cent. In another review, Auerbach and Guggenheim² found 29 such cases among 10,165 autopsies of patients with tuberculosis or an incidence of 0.28 per cent. The latter authors were able to collect only 93 cases of nodular myocardial tuberculosis to which they added two cases of their own. Since then, only five other case reports have been found in the English literature, the last report appearing in August 1947.⁷

Three pathologic types of tuberculosis of the myocardium are described: miliary, nodular and diffuse infiltrating. (1) The *miliary* form is said to have no particular site of predilection. The nodules are 1 to 3 mm. in diameter, yellowish gray to gray in color, and microscopically are characterized by local proliferation of epithelioid cells surrounded by a zone of lymphocytes with displacement of adjacent muscle fibers and only slight degenerative changes in the surrounding myocardium. Usually, there are few giant cells and sometimes there are none. There appears to be no agreement as to the frequency of miliary tuberculous myocarditis in generalized miliary tuberculosis. Various reports give figures ranging from 15 to 100 per cent. (2) In the *nodular* form of tuberculosis, of which our case is an example, the nodules have been found to measure from 5 mm. to 7 cm. in diameter. The nodules are white or yellowish white, have a firm consistency, may be round or oval and present an irregular surface. Microscopically, the picture is that of a central area of caseation surrounded by a connective tissue capsule. The capsule as well as the adjacent myocardium is infiltrated by lymphocytes which are mingled with epithelioid cells, the latter being more numerous as the area of caseation is approached. Giant cells are generally present but may be absent. (3) The *diffuse infiltrative* type of tuberculous myocarditis is grossly characterized by uniform gray to yellowish gray tissue which appears to replace almost entirely the cardiac musculature in the involved portions. Histologically, the usual picture consists of granulation tissue with epithelioid cells, giant cells and lymphocytes.

The following case is an example of the nodular form of myocardial tuberculosis which is of special interest, not only because of its rarity, but because it is ap-

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parently the first reported case in which this lesion was found following prolonged treatment with streptomycin.

REPORT OF CASE

Clinical Data

A 21 year old white man was admitted to Dearborn Veterans Administration Hospital on March 28, 1947, with a history of vague illness and excessive loss of weight, beginning in May 1946. In January 1947, x-ray films of the chest revealed evidence of moderate left pleural effusion, soft patchy infiltrative lesions in the right apex with prominent trunk markings extending into the hilum, a small patchy infiltrative lesion in the lateral portion of the right lower lung field and similar changes in the left infraclavicular region. On March 1, 1947, severe headache and nuchal rigidity developed and spinal fluid examinations shortly thereafter revealed evidence of tuberculous meningitis. Starting on March 13, streptomycin, 2 Gm. intramuscularly, was given daily in divided doses and was continued up to the day of admission to this hospital. Physical examination on admission revealed a markedly undernourished, chronically ill patient with moderate mental confusion, nuchal rigidity and indefinite changes in reflexes. The blood pressure was 118/86 and the pulse rate 80 per minute. Examination of the heart was negative. X-ray films of the chest, on March 31, showed no evidence of pleural fluid and considerable clearing of the previously described infiltrations. Streptomycin was continued in doses of 0.25 Gm. intramuscularly every three hours and 0.1 Gm. intrathecally once daily, until June 2, by which time the patient had received a total of 171 Gm. The temperature ranged between normal and 102 F., while the pulse rate per minute usually varied between 80 and 120.

From July 15 until the patient's death the pulse rate tended to remain rapid, between 150 and 160 per minute. Except for a gradual fall in blood pressure during the last days of life, no significant cardiac alterations were noted. At no time was the rhythm found to be irregular. The patient expired in coma on July 22, 1947, with evidence of greatly increased intracranial pressure.

Autopsy Findings

Gross findings. Autopsy was performed five and one-half hours after death. Severe emaciation was noted. The finger nail beds were cyanotic. Upon opening the chest cavity the pericardial sac was found to be free of adhesions. It was lined by a smooth, glistening, translucent serosa and contained about 60 cc. of slightly greenish yellow clear fluid. The visceral pericardium was also smooth and glistening. The heart weighed 180 Gm. On section, the right ventricle was 3 mm. and the left ventricle, 11 mm. thick. The coronary arteries and the valve leaflets were normal and the myocardium was pale reddish brown. In the interauricular septum and projecting into the right atrium there was a raised, rounded, pale, yellowish white mass 1.5 cm. in diameter with a flat, somewhat irregular surface (Fig. 1). On the left auricular aspect of the septum the nodule produced an irregular depression 1.3 cm. in diameter in the center of which was a yellow, rounded projection 4 mm. in diameter, which was elevated about 1 mm. above the surrounding depression. On section the nodule contained grayish yellow, moderately firm, caseous material in smears of which rare acid-fast bacteria were demonstrated after appropriate staining.

The right lung lay free in the pleural cavity. There were easily broken adhesions between the middle and lower lobes. An emphysematous bleb, 4 mm.

in diameter, was found subpleurally in the apex. In both the upper and lower lobes there was a single grayish white caseous nodule, each 3 mm. in diameter. The left lung showed a generalized fibrinous pleuritis and, on section, evidence of acute passive congestion and patchy areas of consolidation in the lower lobe. An area of caseation 1.5 cm. in diameter was present adjacent to a bronchus. Two hilar lymph nodes located posterior and superior to the left main stem bronchus measured 2 cm. and 2.5 cm. in diameter, respectively, and showed extensive caseation necrosis. The liver was slightly enlarged but free of tuberculous lesions. The spleen was normal. The right kidney revealed one, and the left kidney, four subcapsular miliary tubercles.



FIG. 1. TUBERCULOUS NODULE IN INTERAURICULAR SEPTUM AS SEEN FROM RIGHT AURICLE.

The dura was normal. The brain weighed 1540 Gm. and showed flattening of the convolutions and a pressure ring on the inferior surface of the cerebellum. The base of the brain and the spinal cord were covered by a gray membrane which hid the finer markings and measured up to 4 mm. in thickness. Miliary gray nodules were noted in the thickened pia-arachnoid covering the cerebellum. In cut sections, yellowish gray areas of thickening, 3 mm. in diameter, were present in both lateral cerebral fissures, some areas on the left side tending to be confluent. Only two nodules were found in the cortex of the medial surface of the left temporal lobe. The entire ventricular system was considerably dilated and had a finely granular lining.

Microscopic findings. The nodule in the interauricular septum was surrounded

by a thin fibrous capsule and consisted almost entirely of dense caseous material. It was entirely within the myocardium, although it reached to within 75 microns of the endocardial surface at one point. A thin zone of lymphocytes surrounded most of the mass and no giant cells were seen. Sections from other portions of the myocardium were normal.

Sections of the lungs revealed caseation necrosis and early bronchopneumonia. Caseous tuberculosis of the peribronchial lymph nodes was verified. The pia-arachnoid in the lateral fissures of the brain was involved in an active caseous tuberculosis. Miliary tubercles were found in the ventricular lining and in the choroid plexus. Although there was an active tuberculosis involving the meninges of the spinal cord, it was unusual in that many of the small arterioles in the subarachnoid space were closely bordered by collars of lymphocytes and epithelioid cells, which in turn were surrounded by caseation necrosis. The vessels showed tuberculous endarteritis. In sections stained by the Ziehl-Neelsen technic many acid-fast bacilli singly and in clumps were found in the caseous areas in the cerebrum. A few acid-fast granules were found in the sections of the lungs, but neither acid-fast bacilli nor granules were found in sections of the tuberculoma of the myocardium.

Anatomic diagnoses. Tuberculous meningitis with internal hydrocephalus; nodular caseous tuberculosis of lower lobe of left lung; nodular tuberculosis of upper and lower lobes of right lung; caseous tuberculosis of peribronchial lymph nodes; *tuberculoma of interauricular septum of heart*; miliary tuberculosis of kidneys; chronic cystitis; fatty metamorphosis and passive congestion of liver; moderate hyperplasia of spleen.

DISCUSSION

In a review of 72 cases of tuberculosis of the heart by Anders,¹ the wall of the right auricle was found to be the most common site of nodular tuberculosis. Less frequently involved were the left auricle, left ventricle and right ventricle in the order given. Carson,⁴ in his review of 87 cases, found the right ventricle most frequently involved and next most frequently, the right auricle. According to more recent reviews,^{2, 5} it is generally agreed that the right side of the heart shows a greater incidence of lesions, particularly the wall of the right auricle. Involvement of the interauricular septum without involvement of other parts of the myocardium has been rarely described.

The routes by which the myocardium is involved have intrigued many authors but no one has presented conclusive evidence for any single pathway. Direct extension accounts for many instances since many lesions are contiguous with tuberculous involvement of the pericardium. Among the 72 cases reviewed by Anders,¹ in 31 there was definitely associated tuberculous pericarditis, in 31 cases such associated pericarditis was uncertain, and in only 10 cases was it stated that there was no pericardial disease. The hematogenous route by the coronary arteries and possibly the intracardiac blood stream may explain a miliary spread but it is difficult to conceive of a blood-borne infection as the cause of a single large tuberculoma. The lymphatic route is favored by most

writers and seems the most logical explanation for the pathogenesis of isolated tuberculomas. Kampmeier⁶ pointed out that in the heart of the 3.8 month old fetus, which he stated is comparable to the adult heart, there are only four or five valves in the lymphatics of both atria as compared to more than 300 in the ventricles. He also pointed out that distention of the major vessels in cardiac systole and the suction produced by the relaxing myocardium in diastole promotes the retrograde flow of lymph. This flow is prevented only by the presence of the small number of lymphatic valves. This finding, plus the demonstration by von Recklinghausen⁸ that retrograde lymphatic flow actually does occur in the abdomen, point strongly to spread by way of the lymphatics. The lymphatics that drain the right ventricle and auricle empty into a trunk which flows into the anterior mediastinal group of lymph nodes situated in the adult at the root of the left common carotid artery. Tuberculous nodes at this site probably act as the source of the organisms which eventually invade the myocardium. Despite the natural resistance of the myocardium to tuberculosis, and despite the administration of streptomycin to our patient over a long period of time, the growth of the tuberculoma was not inhibited. We believe it is safe to assume that the myocardial lesion existed prior to the beginning of streptomycin therapy, but it was surprising to find that it showed no evidences of healing, such as absence of caseation, fibrosis and hyalinization, which Baggenstoss *et al.*³ described in their recent paper. They suggested, however, that larger lesions show no evidence of regression because an effective concentration of streptomycin fails to penetrate the necrotic mass. This explanation could account for the lack of response in our case. In addition our patient received no streptomycin during the last seven weeks of life.

SUMMARY

A case report is presented of tuberculoma of the myocardium of the interauricular septum in a patient with tuberculous meningitis treated with streptomycin. The incidence, etiology and pathogenesis of this lesion are briefly discussed.

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CLINICOPATHOLOGIC CONFERENCE*

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The patient was a 41 year old white man who was admitted to the Nervous and Mental Service of the hospital on April 13, 1943, and expired on May 19, 1943.

Previous history. He was first admitted to this hospital on January 10, 1943, in an unconscious condition, having been in an automobile accident. He regained consciousness soon after admission and then complained of lethargy and of severe headache. The past history revealed that the patient had acute arthritis at the age of 30, high blood pressure for an indefinite period of time, and a stroke two years previously with temporary paralysis of his left side.

Physical examination revealed a blood pressure of 220/160. A soft systolic murmur was heard at the mitral area, and the area of cardiac dullness extended 12 cm. to the left of the midline. The left hand was swollen and the left arm and hand were spastic. Triceps reflexes were normal and the biceps reflexes were slightly hyperactive bilaterally. Hoffmann's sign was absent, the patellar reflex was graded 1 plus on the right side and 2 plus on the left. The Achilles tendon reflex and the cremasteric reflexes were normal. The abdominal reflexes were bilaterally absent; Babinski's sign was absent on the right and present on the left side. Oppenheim's and Chaddock's signs were absent on the right side and present on the left. The urine contained 2 plus albumin, 20 to 25 red blood cells and occasional pus cells. X-ray films of the skull were negative. A spinal puncture yielded slightly cloudy fluid which contained 55 mg. of protein per 100 ml. and two cells per cu. mm. The red blood cell count was 3,950,000. The blood sugar was 80 mg. and the urea nitrogen 94.5 mg. per 100 ml. The patient continued to complain of headache and backache and some stiffness of the neck. He appeared to become more spastic on the left side and developed a left foot drop. He was transferred to a sanitarium and discharged after fifty-nine days with a diagnosis of malignant hypertension, cerebral hemorrhage with subarachnoid hemorrhage and left-sided hemiplegia.

Present illness. The patient was admitted in an unconscious state on April 13, 1943. The informant stated that the patient had had a generalized convulsive seizure and suddenly collapsed. He regained consciousness but shortly thereafter had another convulsive seizure whereupon he was admitted to the hospital. The informant stated that there were frequent recurrent twitchings and stiffening of the body, and that the respirations had been labored and jerky.

Physical examination revealed a blood pressure of 240/150, a pulse rate of 110, a temperature of 101.2 F. and a respiratory rate of 22 per minute. The patient's face was flushed, there were evident twitchings of the arms and legs, rolling of the

* Received for publication, November 5, 1947.

eyes and wrinkling of the eyelids. The muscular twitchings were quite generalized and of the Jacksonian type but seemed to be localized at times in the left arm and leg. The pupils were equal, regular and reacted well to light; there was lateral nystagmus. A bruised swollen area was present over the left supra-orbital ridge. The ears, mouth and neck were negative. Examination of the heart revealed a moderate left ventricular enlargement; the apical beat was quite prominent and was felt in the sixth interspace just to the left of the midclavicular line. No murmurs were heard; the aortic second sound was louder than the pulmonary second sound. There was atrophy of the muscles of the left arm and leg with plantar flexion and internal rotation of the left foot. The left arm was weak, but the right was held rigidly in a flexed position. The biceps and triceps reflexes were graded 1 plus on the right side and the patellar and Achilles reflexes 2 plus. Babinski's, Oppenheim's, Chaddock's and Hoffmann's signs were present bilaterally. The biceps, triceps and extensor reflexes were graded 2 plus hyperactive on the left, the patellar and Achilles reflexes, 3 plus.

Laboratory findings. The serology was negative. The hemoglobin was 49 per cent; the leukocyte count was 11,250 with 83 per cent neutrophils, 15 per cent lymphocytes and 2 per cent monocytes. The specific gravity of the urine was 1.012, and at no time was it more than 1.013. The urine consistently showed between 1 and 4 plus albumin, and on every examination contained numerous erythrocytes and pus cells.

Hospital course. Soon after admission a phlebotomy was done and 500 cc. of blood was removed, but the convulsive seizures continued. He was given sedation with magnesium sulfate after which the convulsions soon stopped. The blood pressure after the convulsions ceased was 180/110. An electrocardiogram taken on April 26 showed a left axis deviation with depression of ST₁ and diphasic T₁. Funduscopic examination showed marked hypertensive arteriolar changes and a left homonymous hemianopsia. An x-ray film of the chest was negative. On April 19, the blood urea nitrogen was 91 mg., the creatinine 6.8 mg. per 100 ml., and on subsequent determinations these values remained essentially unchanged. Concentration tests of the urine showed an inability to concentrate above 1.010. A spinal tap was done on May 3, and the pressure was 180; the fluid was colorless, contained no cells and the Wassermann test was negative. The temperature remained normal until May 8, at which time it rose to 101.8 F. and remained elevated from that time until he expired. He continued to have occasional twitchings of the extremities. Spinal puncture on May 8, revealed a pressure of 206 mm. (of spinal fluid), which was lowered to 140. The fluid was cloudy, contained 103 mg. protein per 100 ml., a cell count of 620 per cu. mm. with 77 per cent neutrophils and 23 per cent mononuclears. He had a red blood cell count of 4,800,000. The patient became stuporous and then lapsed into coma for about one week. On May 17, he had a Jacksonian epileptic seizure with generalized contractions. He was given sodium phenobarbital and magnesium sulfate intravenously. A spinal tap was again done and the pressure was 220 mm. of spinal fluid, which was lowered to 150 mm. The convulsive seizures continued and he had to be given 200 cc. of 50 per cent glucose, and finally intra-

venous sodium amytal before the convulsive seizures ceased. He remained comatose and expired on May 19, 1943.

AUTOPSY FINDINGS

There was no edema and no ascites but the right pleural cavity contained 1000 cc. and the left 300 cc. of cloudy fluid. The heart weighed 540 Gm. and exhibited left ventricular hypertrophy. The coronary valves and mural endocardium were normal. The right lung weighed 800 Gm. and showed confluent bronchopneumonia. The spleen weighed 140 Gm. and the liver 1900 Gm. and showed no disease. The right kidney weighed 820 Gm. and the left kidney 1240 Gm. Both were largely replaced by numerous large cysts. There was an area of old softening in the right parietal lobe of the brain, measuring 7 x 2 x 3 cm. There was also an area of fresh hemorrhagic softening 2 cm. in diameter in the left basal nuclei, and an old area of softening, 2.5 cm. in diameter, in the right inferior portion of the cerebellum.

Microscopically the areas of renal parenchyma between the cysts showed severe arterial and arteriolar sclerosis with hyalinization of most of the glomeruli and extensive tubular atrophy.

The pathologic diagnoses were polycystic renal disease with severe renal arteriosclerosis and atrophy, primary hypertension with cardiac hypertrophy, old encephalomalacia of the right parietal lobe and right hemisphere of the cerebellum, and fresh hemorrhagic softening in the left basal nuclei.

DISCUSSION

The enlarged kidneys were overlooked clinically, probably because the case was typical for primary hypertension with chronic uremia, and this diagnosis was correct. This is a rare combination of primary hypertension and polycystic kidneys. It will be noted that the blood urea nitrogen remained about 90 mg. per 100 ml. throughout the period of observation, which is unusual in primary hypertension but frequent in polycystic renal disease. A diagnosis of primary hypertension is suggested by the apoplectic strokes rather than a diagnosis of chronic glomerulonephritis in which such strokes are rarely seen. The lesion in the right parietal lobe evidently extended deeply into the brain and cut the optic radiation on that side to produce the hemianopsia.

EDITORIALS

BLOOD BANKING AND THE CLINICAL PATHOLOGIST

A recent Blood Bank Institute held at Dallas was attended by about 125 medical and nonmedical people interested in blood bank operation from all parts of this country, as well as Hawaii and Mexico. Acting in the capacity of hosts were Dr. Joseph M. Hill and his group at Baylor Hospital. The program was very well arranged and hospitality and efficiency characterized the whole meeting. Although the original purpose of the Blood Bank Institute was for the presentation of methods of establishing and operating blood banks, and for the teaching of new technics for processing blood for transfusions, it soon became evident that those registering for the Institute were also interested in the formation of an association of blood banks.

As the result of unanimous sentiment expressed, the American Association of Blood Banks was formed for the purpose of uniting ethical and independent banks throughout the country and rendering "useful service to all communities or hospitals and to those persons throughout the United States and its territories who need or will need whole blood or its derivatives". This association contemplates the elevation of uniform standards by cooperative and educational methods, the encouragement of hospital and community blood bank formation and the formulation of plans for cooperation in times of disaster.

The resolutions adopted by the Association were definitely intended to put blood bank problems on a local level with the medical profession by way of the county societies acting in an advisory capacity. Through this approach each organized blood bank could serve the medical profession and the community on the basis of local conditions, demands and patient needs.

It is very evident from the success of community, hospital, and county medical society supported blood banks that this service can be increased adequately to provide blood to the population of entire states. Such an expansion can be accomplished through the independent blood bank in an economical fashion without any drain on charity or State and Federal funds. By this approach we can continue to maintain the intimate patient-physician relationship that has been so successful in our country. Furthermore, under the independent blood bank, professional services and consultation follow the blood to the patient. There seems to be no more reason for the socialization of blood banks than of the practice of medicine in general or that of clinical pathology in particular.

Two important premises entered into the organization of this association. The first was that the public and medical profession were better served by blood banks which are enterprises of hospitals or communities, the reason being that more genuine interest is usually generated by actual knowledge and vital contact with an activity of this sort. The second was that it is not only preferable but wise to have blood replacements made by relatives and friends of the recipient rather than by volunteer donors, the reason for this being the value to the

patient and the family of the development of initiative, independence and self-reliance which the exertion and effort of repayment necessitates.

About one-third of those registered at the Dallas meeting were pathologists, the remainder being interested from an administrative or technical viewpoint. While blood bank administration is recognized as important, there seems to be a conspicuous tendency on the part of pathologists to turn blood bank operation and control over to others. The indifference of most clinical pathologists to this very important phase of medical care is not in the best interests of the future of clinical pathology. Blood banking is logically a laboratory activity. Directors of blood banks should be pathologists. Future meetings having to do with the problems of blood banking should be attended predominantly by pathologists.

The field of clinical pathology is being constantly encroached upon by our nonmedical friends, the bacteriologist and the medical chemist. Laboratory crime-detection is not being fully developed by the pathologist. Clinical and public health laboratories are frequently operated and directed by our confreres in other fields and specialties. And now is the development and direction of blood banks to pass into other hands?

A complete and aggressive appreciation, exploration and exploitation of the opportunities of our specialty will lead to a broadening of our field of endeavor and to increasing the attractiveness of pathology to medical students and recent graduates seeking a suitable specialty—other than surgery.

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THE HISTOPLASMIN SKIN TEST

The histoplasmin skin test has come into prominence recently due to reports of a relationship between histoplasmin sensitivity and pulmonary calcification as seen in chest roentgenograms among tuberculin-negative persons.^{2,6} This association between histoplasmin sensitivity and pulmonary calcification has been questioned by some workers⁵ but confirmed by others.^{7,10} The bulk of the evidence strongly favors the relationship between histoplasmin sensitivity and pulmonary calcifications in tuberculin-negative persons.

Independent of the association between histoplasmin sensitivity and pulmonary calcifications, there is the question of the significance of histoplasmin sensitivity. Although histoplasmin is a filtrate of a broth culture of *Histoplasma capsulatum*, there has been some reluctance to accept a skin reaction to the antigen as evidence of infection with this fungus. The reason for this reluctance is the indirect nature of the evidence linking histoplasmin sensitivity with histoplasmosis. In experimentally infected guinea pigs, it was observed by Olson, Bell and Emmons³ that histoplasmin sensitivity occurred in animals infected with *Blastomyces*, *Coccidioides*, *Haplosporangium* and *Candida*. The most frequent cross reactions were seen among the animals infected with *Blastomyces*. These authors concluded that histoplasmin sensitivity, at least in animals, may be due to infection with any one of the above group of fungi.

In another recent article⁴ on the results of similar animal experiments to determine the specificity of the histoplasmin test, it was concluded that the frequency of cross reactions between these antigens was small if proper attention was paid to the selection of the dose of antigen used. Further, each antigen caused reactions far more frequently in guinea pigs infected with the homologous organism than with the heterologous organism. Large numbers of animals and antigens prepared from both the mycelial and yeast phase of *Histoplasma* and *Blastomyces* were used in these experiments. It thus appears that, while cross reactions may occur, their significance is not clear from animal experiments.

From a clinical standpoint it may be noted that only a few proved cases of histoplasmosis have been tested with histoplasmin. Of 8 cases reported prior to May 1947, only three persons reacted. Of the five negative reactors, four were skin tested within one month of death and hence may have been in the anergic phase. In a recent report¹ of four additional proved cases, all of the patients were sensitive to histoplasmin.

It is interesting to note, however, that three of these four patients did not react to the first histoplasmin test although they did to numerous later tests. It appears, therefore, that repeated tests are necessary in any given instance to be certain that the patient does not react.

A newly developed⁹ complement-fixation test using histoplasmin as the antigen supports the view that histoplasmin is specific. More complement-fixing antibodies against histoplasmin than against blastomycin were found in the serums of guinea pigs infected with *Histoplasma capsulatum*; the reverse was true in the serums of guinea pigs infected with *Blastomyces dermatitidis*. Positive complement-fixation tests have been reported in four persons with proved histoplasmosis¹ as well as in a number of persons with active lung lesions associated with histoplasmin sensitivity.⁸ Persons with healed lung lesions, or no lung lesions, gave negative complement-fixation tests even though they were histoplasmin-sensitive. Histoplasmin-negative persons showed no fixation.

In summary, it appears that histoplasmin sensitivity is probably an indication of present or past infection with *Histoplasma capsulatum*. The test should be repeated in suspected cases in persons who are seriously ill or have a fever and do not react.

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SELECTED ABSTRACTS

Cancer in the Medical School Curriculum. THE NATIONAL ADVISORY CANCER COUNCIL. J. Nat. Cancer Inst., 8: 1-6, 1947.

Upon the recommendation of the National Advisory Cancer Council a Committee on Cancer in the Medical School Curriculum has been formed. This committee, composed of twenty-two deans and professors from fourteen medical schools, met in November 1946. The underlying motivation of this conference was the realization that the major defect in effective cancer control is delay in its detection. Present treatment methods are more efficient than methods for the early recognition of cancer. This situation is often due to lack of alertness and interest in cancer on the part of the medical profession, more especially the family physician. The committee considered that the place to initiate interest in and awareness of cancer is in the medical school. Undergraduate cancer teaching is for the most part inadequate, not well coordinated, and the fundamental biology of cancer is usually neglected.

The outgrowth of the discussion based on a profound respect for the importance of this disease and the vital role of the medical profession in its control was the recommendation that a vertical scheme of cancer teaching, particularly in the third and fourth years, supplement the horizontal plan now in effect. Such an effort would necessitate additional personnel and facilities and it was felt that it might be desirable to establish in medical schools a chair of oncology to be held by a member of the teaching staff with broad clinical experience in cancer. In the capacity of director or chairman at professorial level this individual would be in a position to organize, supervise and coordinate a teaching plan in the various departments concerned.

The vertical plan might include: (a) study of physiology and biochemistry of cancer and cancerogenesis; (b) a review of the pathology of neoplasms; (c) study of clinical cancer in cancer clinics; (d) use of cancer detection clinics to increase the thorough examination of supposedly well people and recognition of potentially cancerous lesions as a means of cancer prevention; (e) use and evaluation of newer laboratory diagnostic procedures in cancer, e.g., smear diagnosis; (f) the additional use of seminars, conferences and journal clubs to coordinate cancer clinic activities with biology and research and to stimulate interest in the current medical literature on the subject.

The important role of the department of internal medicine in the program is becoming appreciated. An interdepartmental cancer committee in each school is necessary to integrate all phases of the plan. Part of the undergraduate curriculum might be made elective and interwoven with the graduate program in the teaching of residents and with a cancer institute type of program for physicians. The stimulation of an interest in cancer research at the undergraduate level should be an important objective and could be aided by an active research program in the school.

The final recommendation of the committee was that the United States Public Health Service consider ways and means for providing financial assistance to the medical schools interested in undertaking such a plan of cancer teaching. This has been forthcoming and is now available.

The philosophy underlying the deliberations and recommendations of the committee is that, aside from the concrete information imparted to students, a discipline and an atmosphere be created in medical schools which would produce lasting impressions on the minds of the students, leading to an awareness and keenness of perception applicable to the clinic and office examining rooms of future specialists and general practitioners. A genuine interest in basic cancer investigation might also be stimulated.

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Congenital Cysts and Fistulae about the Head and Neck. CHARLES D. BLASSINGAME. Ann. Otol., Rhin. and Laryng., 56: 395-403, 1947.

Early workers believed that lateral cysts and fistulas of the neck were of branchial arch

origin. In 1913, Wenglowski presented the theory that these congenital defects were more probably related to the embryologic development of the thymus. The thymic bud originates as a diverticulum at the bottom of the third pharyngeal pouch. At birth the thymus lies behind the sternum. This difference in position occurs as a result of the formation of the neck and the descent of the heart during embryonic life. The path over which the thymus moves from point of origin to the point at which it is found at birth is known as the thymic corridor.

To support the idea that lateral cysts and fistulas were remnants of the thymic corridor, Wenglowski dissected 65 embryos and 10 adult bodies. The course of the thymic corridor was found to be laterally from the pharynx to a point between the angle of the jaw and the lobe of the ear, then downward and medially along the lateral lobe of the thyroid. It passes posterior to the belly of the digastric muscle and anterior but very close to the carotid sheath. He also found that the lower half of the corridor persists more frequently than the upper. External openings of this corridor were seen on the skin surfaces, usually along the anterior border of the sternocleidomastoid muscle. Remnants of thymic tissue were found along the corridor in 21 of the 65 embryos and 2 of the 10 adult bodies.

The author presents a case history of a 7 year old girl with a draining skin sinus which had persisted from soon after birth. The sinus opened on the skin surface on the right side just above the sternal notch. At operation the sinus tract was found to pass cephalad along the lateral lobe of the thyroid on the right, then medially toward the pharynx. Methylene blue solution injected into the sinus tract appeared on the surface of the right faucial tonsil.

The experience and investigations of this author suggest that the theory of thymic origins of lateral cysts and fistulas corresponds with known embryologic facts as well as with clinical experience. This theory will be of interest to the pathologist who occasionally encounters these lesions and to the surgeon because of the therapeutic implications involved.

O. A. BRINES

Oral Emetine in the Treatment of Intestinal Amebiasis. BLISS C. SHRAPNEL. *Am. J. Trop. Med.*, 27: 527-544, 1947.

Although emetine is a powerful amebicide, because of its irritating effect on the stomach resulting in nausea and vomiting, it is used parenterally and only for extra-intestinal amebiasis. Even parenterally it must be administered with caution because of its toxic effects, particularly on the myocardium. It is surprising to find only a few recorded instances of attempts to coat the drug with substances insoluble in the stomach.

The author used emetine hydrochloride "Enseals" (Enteric Sealed Tablets, Lilly) given orally, two grains a day for twelve days for adults and one grain a day for twelve days for children. Clinical cure was obtained in all instances (25 adults and 5 children). Parasitologic cure failed in only 1 patient, an adult who was found to harbor *E. histolytica* trophozoites seven months after receiving the drug. This, however, could have been a reinfection.

The only evidence of toxicity observed in the series was a mild irritative effect on the intestine resulting in two or three soft stools a day, without cramps or tenesmus. There were no changes in blood pressure, pulse or electrocardiograms, no urinary changes, and no joint pain, which is a symptom often seen when emetine is given hypodermically.

In addition to the effect on amebae it was noted that 2 children in this series who were heavily infested with *Trichuris trichiura*, passed large numbers of these worms in their early morning stools. The author believes that controlled studies on the effect of emetine in this parasite would be of value.

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Traumatic Rupture of Bronchus. T. J. KINSELLA AND L. W. JOHNSRUD. *J. Thoracic Surg.*, 16: 571-583, 1947.

The authors report 2 cases of traumatic bronchial rupture, the first of which, a 15 year

old boy, was seen two and one-half years after a wagon wheel partly mounted his chest. Respiratory difficulty necessitated pneumonectomy. The excised lung was completely atelectatic and had suffered tears of the main and upper lobe bronchus with healing and complete obstruction. Microscopically, there was "diffuse congestion with thickening" of the lung.

The second case was that of a 7 year old boy who was crushed laterally between truck and brick walls. Bronchoscopic examination uncovered a tear of the main bronchus. Two months later the bronchial lumen was markedly stenotic, and the lung completely atelectatic. After one and one-half years, the condition was unchanged.

Permanent pulmonary atelectasis, secondary to bronchial obstruction, may be the sequel to tearing of the bronchus. A nonfatal outcome is recorded in one-half of the 38 cases collected from the literature.

Fort Wayne, Indiana

S. M. RABSON

An Epidemic of Diarrhea in a Newborn Nursery Caused by Pseudomonas aeruginosa. C. A. HUNTER AND P. R. ENSIGN. *Am. J. Pub. Health*, 37: 1166-1169, 1947.

The milk supply of a dairy was contaminated by *Ps. aeruginosa*. This organism gained entrance to the milk through water dripping from a rag. The contaminated milk evoked an epidemic of gastro-enteritis and caused outbreaks among patients and employees of the hospital of Great Bend, Kansas, who secondarily infected infants in the nursery. Twenty-four newborn infants became ill; 9 of them died. Therapeutically, penicillin, transfusions and sulfa drugs had little effect, while methylene blue, used on a hypothetical basis, to counteract the small amounts of hydrocyanic acid formed by some strains of *Ps. aeruginosa*, was very useful in the cases in which it was given.

This paper is a valuable contribution to the problem of diarrhea in the newborn and reports the results of a careful epidemiologic study.

San Juan, Puerto Rico

OSCAR FELSENFELD

A Study of the Postmortem Bone Marrow from Cholera Cases. H. N. CHATTERJEE. *Trans. Roy. Soc. Trop. Med. and Hyg.*, 40: 905-908, 1947.

Fifteen cases are reported in which the bone marrow from femur, tibia and humerus was studied. An acute dilatation and engorgement of the system of capillaries of the bone marrow was found. These capillary changes were more marked in the bone marrow than in any other organ. The author believes that this might at least partly explain the great shock so often observed in cholera. The sinusoids were also distended. Eosinophilia and a variable amount of leukoblastic reaction were regularly present. Small lymphatic nodules have been seen in some cases.

This paper represents a continuation of the author's previous studies of the bone marrow in cholera and is accompanied by good illustrations.

OSCAR FELSENFELD

Studies on Experimental Goitre. VIII. Thyroid Tumours in Rats Treated with Thiourea. H. D. PURVES AND W. E. GRIESBACH, *Brit. J. Exper. Path.*, 28: 46-53, 1947.

Thiourea was administered to rats in drinking water for prolonged periods. After one year of this treatment, adenomas of the thyroid had developed in 80 per cent of the animals. In one group of rats the administration of the drug was continued for a period of 20 months or longer. Of the 10 tumors developing among these animals, 7 proved to be malignant, showing the histologic picture of adenocarcinoma of the thyroid. Invasion of blood vessels by neoplastic elements was regularly noted, and in two instances, pulmonary metastases occurred.

The considerable stimulation of proliferation in the human thyroid which may result from thiouracil medication has become well known during the last years. In this connection, it may be well to bear in mind that under certain conditions, such as described

above and also in an older study of Bielschowsky (1944), substances closely related chemically to thiouracil proved carcinogenic in animal experiments.

Chicago

KURT STERN

Congenital Defects in a Year of Epidemic Rubella. R. E. OBER, J. M. HORTON AND R. F. FEEMSTER. *Am. J. Pub. Health*, 37: 1328-1333, 1947.

The authors report 54 new cases of rubella occurring during pregnancy, making a total of 78 cases in the recent American literature. Of the 40 cases which occurred during the first trimester, 20 (50 per cent) resulted in abnormalities (13 defective infants and 7 abortions or stillbirths). There were 22 cases occurring during the second trimester with 3 (13.6 per cent) abnormalities (2 defective infants and 1 abortion or stillbirth). During the third trimester, there were 24 cases with 14.3 per cent or 2 abnormalities (2 abortions or stillbirths). Two cases in which the time of occurrence during pregnancy was not known resulted in 1 defective infant (50 per cent). A similar proportion of defective and lost infants has been shown to occur when pregnancy is complicated by poliomyelitis. The need for further similar surveys with other diseases occurring during pregnancy is stressed.

British Anti-lewisite. R. A. PETERS *et al.* *Brit. M. J.*, 1: 520-521, 1947.

BAL, 2,3-dimercaptopropanol, is of distinct value in the treatment of arsenical dermatitis, encephalopathy and granulocytopenia, acute mercury poisoning and gold dermatitis. The work with other heavy metals, such as lead and bismuth is still in the stage of animal experimentation. BAL, in doses of 3-5 mg./Kg. of body weight, is felt to be toxic, causing lacrimation, salivation, vomiting and an elevation of the blood pressure. Renal damage in animals has not been found to increase its toxicity but increased toxic effects have been noted in animals with liver damage. Since postarsphenamine hepatitis is infective in most cases, BAL is not of value in its treatment.

BOOK REVIEWS

Gynecological and Obstetrical Pathology with Clinical and Endocrine Relations. Ed. 2. By EMIL NOVAK, M. D., D.Sc., Associate in Gynecology, Johns Hopkins Medical School; Gynecologist, Bon Secours and St. Agnes Hospitals, Baltimore. 570 pp., 542 illus., 15 in color. \$7.50. Philadelphia: W. B. Saunders Company, 1947.

This new edition of an extremely popular book will be received with continued enthusiasm by all students of gynecologic and obstetric pathology, whether obstetrician, gynecologist, or pathologist. The first edition brought together for the first time in the English language, the modern authoritative views of gynecologic and obstetric pathology and so filled an important need. So much had transpired in the preceding decade in the field of gynecologic research that a real necessity existed for a book which would bring these advances under one cover. These researches in endocrinology, chemistry, cellular pathology and oncology had the effect of revolutionizing the practice of gynecology. Novak accomplished the collection of this information in a masterful manner, which reflected his experiences as a foremost practitioner of obstetrics and gynecology, and only slightly less his ability as a gynecologic pathologist.

The second edition follows the pattern of the first but is somewhat larger, comprising 570 pages with 542 illustrations. More than 100 illustrations are new and 15 of these are in color. The added illustrations make, as Novak states, "a profusion of illustrations". The work is concise and well organized and adequate for practical everyday needs although suffering from brevity for those interested in a comprehensive book.

Generally, there are no suggestions for improving the text except perhaps in the chapter on Cystic Changes (Chapter VII), which the reviewer believes could be brought into line with more commonly used and simplified nomenclature, so far as it applies to the classification of cyclic endometrial changes. The section on obstetric pathology might also be expanded.

The pathologist and those specializing in obstetrics and gynecology will derive satisfaction from this edition of Novak's *Obstetrical and Gynecological Pathology*, even more than from the first edition, since sufficient new material has been added to make it a real contribution to the subject.

Detroit

D. C. BEAVER

Diagnostic Bacteriology. Ed. 3. By ISABELLE GILBERT SCHAUB, A. B., Instructor in Bacteriology, Department of Bacteriology, The Johns Hopkins University School of Medicine; and M. KATHLEEN FOLEY, A. B., Instructor in Bacteriology, Department of Biological Sciences, College of Notre Dame of Maryland. 532 pp., 21 tables. \$4.50. St. Louis: The C. V. Mosby Company, 1947.

There probably can be no better evidence of the practicability of this book than the fact that, although it was first published in 1940, it is now in its third edition. It should be emphasized, however, that in spite of the change in title from *Methods for Diagnostic Bacteriology*, the book remains essentially a manual in diagnostic bacteriology. No manual can completely satisfy everyone in terms of format, order of presentation, procedures included or procedures excluded. The reviewer considers the left hand blank page to be serviceable for notes and/or revisions. At times there is difficulty in following a given procedure to its logical completion without reference to the index. In many instances, it was felt that the presentation would have been clearer had pertinent serologic procedures been included with the primary procedure, rather than incorporated in a separate inclusive chapter on serology. It should be emphasized, as the authors have done, that selection of a given procedure for inclusion in a manual is the end result of accumulated experience in a given laboratory and that other procedures and other methods can and do yield equally satisfactory results.

In the field of enteric bacteriology, particularly, although also to some extent in other specific procedures, the methods as outlined seem to emphasize unduly the importance of complete biochemical analysis. For example, the authors give a method for identification of *Salmonella* species based entirely on biochemical reactions. By international agreement identification of *Salmonella* is based on antigenic analysis. Furthermore, valuable time in reporting results can be saved in many instances by early recourse to serologic means of identification. Any laboratory in which accurate and adequate serologic reagents are available can isolate and identify by species, *Shigella* within forty-eight hours, typhoid within forty-eight or seventy-two hours, and an organism as one of the *Salmonella* within the same working period. We would also suggest the advisability of rapid identification of *H. pertussis* by means of specific serums rather than by the slower biochemical methods advocated by the authors. Although the authors give specific sources for diagnostic anti-serums, the reviewer must note that there does not appear to be a commercial source of antisera at this moment for the typing of pneumococci, the identification and typing of meningococci or the identification of *Klebsiella*. Our information indicates that, unfortunately, neither the sources given in the book nor other commercial sources can supply these antisera at present.

The book is to be recommended to those who seek a manual of diagnostic bacteriologic procedures from which to develop new procedures and to those who wish to review their own procedures; also, as a source of valuable detailed information in the implementation of such procedures.

Lansing, Michigan

H. E. CORE

Bacteriology. Laboratory Directions for Pharmacy Students. Ed. 2. By MILAN NOVAK, Ph.D., M.D., Professor and Head of the Department of Bacteriology and Public Health, and ESTHER MEYER, Ph.G., Ph.D., Assistant Professor of Bacteriology and Public Health, University of Illinois Colleges of Medicine, Dentistry and Pharmacy, Chicago. 247 pp. \$2.75. St. Louis: The C. V. Mosby Company, 1947.

This manual was designed to present laboratory exercises in bacteriology in a systematic way to pharmacy students. The outline gives in detail the mediums, cultures and reagents required for each student, the methods and materials to be demonstrated, and a brief discussion of the work for each day. Adequate space is allowed for drawings, and tables are provided for recording the results of the work performed.

The directions given for 36 laboratory periods cover an adequate laboratory course in bacteriology for the pharmacy student. The experiments embody the necessary procedures for teaching bacteriologic technic and introduce the important pathogenic microorganisms. Exercises such as sterility tests, and assays of bactericidal and fungicidal products, which are of special practical importance to pharmacy students, are included. Each experiment given, however, is so basic that the manual could be used to present introductory laboratory material to students in any of the medical sciences.

Detroit

DANIEL E. HASLEY

De Ziekte Van Besnier-Boeck en bacteriël-allergische ontstekingsprocessen (Besnier-Boeck's Disease, A Bacterial Allergic Infectious Process). By THEODOR GERARD VAN RIJSSEL, M.D. 244 pp., 43 illus. Utrecht: Kemink En Zoon N. V., 1947.

This is a critical study of an important and still obscure problem. To 30 cases reported in the literature the author adds 3 well-studied and well-illustrated cases of his own. The clinical symptomatology and the gross and microscopic morbid anatomy are described in detail. An analysis of the bibliography concerning sarcoidosis as well as granulomata not featuring microorganisms, especially rheumatic nodules, and his own histopathologic observations led the author to suggest that sarcoidosis is an allergic inflammatory lesion. This concept explains the absence of demonstrable microorganisms. In some patients sarcoidosis begins with polyarthritides and sometimes erythema nodosum is present; co-

sinophilia is common and eosinophilic leukocytes are frequently seen in young lesions, myocardial foci resemble Aschoff's nodules; and, finally, in one of the author's patients intimal granulomata were found, such as seen in allergic states. The author concludes that Besnier-Boeck's disease is a form of tuberculosis characterized by energy, that is, a special stage of allergy.

This study and review represent a valuable contribution. While there is an English summary, the Dutch text reads easily.

Wilmington, Delaware

O. J. POLLAK

Pathology of Labor, the Puerperium and the Newborn. Ed. 2. By CHARLES O. McCORMICK, A.B., M.D., F.A.C.S., Clinical Professor of Obstetrics, Indiana University School of Medicine. 514 pp., 272 illus., 24 in color. \$8.50. St. Louis: The C. V. Mosby Company, 1947.

This book contains little that will be of interest to most of the readers of the Journal, since the material included is limited almost exclusively to the clinical aspects of obstetrics.

The greater part of the book is devoted to labor and is discussed under the major headings of abnormal labor, obstetric injuries, postpartum hemorrhage and obstetric operations. A smaller part is devoted to the puerperium; this includes puerperal fever, late postpartum hemorrhage, diseases of the breasts and other complications of the puerperium. The section on the newborn takes up less than 10 per cent of the book and discusses very briefly conditions related to delivery, conditions of congenital origin and conditions peculiar to the newborn period. An appendix includes miscellaneous information concerning analgesia and anesthesia, early puerperal rising, bed posture and exercise, classification of monstrosities, religious restrictions and statistical data.

Chicago

EDITH L. POTTER

Internal Medicine in General Practice. Ed. 2. By ROBERT PRATT McCOMBS, M.D., Assistant Professor of Medicine and Director of Postgraduate Teaching, Tufts College Medical School; Senior Attending Physician, The Joseph H. Pratt Diagnostic Hospital. 697 pp., 122 figs., 15 tables. \$8.00. Philadelphia: W. B. Saunders Company, 1947.

This new edition follows the same plan as the first, discussing the common diseases of the United States and omitting the rare diseases. The book can best be described as an excellent quick reference for the general practitioner who desires to obtain essentials in differential diagnosis and the recent advances of the common conditions encountered in the general practice of medicine.

From the point of view of the clinical pathologist, some exceptions might be made in the discussion of what should be routine laboratory studies. The author suggests a urinalysis, Wassermann test, hemoglobin determination, and examinations of the blood smear, sedimentation rate and stool. While there is no uniformity on this matter, it would seem more practical to use the Kahn test routinely and to include red and white blood cell counts.

The book is well illustrated and has excellent tables, largely on differential diagnosis. The subject matter has been brought up to date particularly with reference to chemotherapy where the use of antibiotics and sulfa drugs are well discussed and the antihistamine drugs evaluated.

Chicago

I. PILOT

NEWS AND NOTICES

COMMITTEE ON NOMENCLATURE FOR HEMATOLOGY

The first meeting of the Committee for Clarification of the Nomenclature of Cells and Diseases of the Blood and Blood-Forming Organs, sponsored by the American Society of Clinical Pathologists and the American Medical Association, was held in Chicago, at The Drake Hotel and at Northwestern University Medical School, October 25 and 26, 1947.

The report of the recommendations of the committee for nomenclature of the cells of the blood and blood-forming organs will be published in a subsequent issue of the American Journal of Clinical Pathology.

It is now being circulated among the 36 members of the committee, including representative hematologists, clinical pathologists, and editors of medical journals in the United States, Canada and Great Britain, for final approval before publication.

COMMITTEE ON TUMOR TERMINOLOGY

The first meeting of the Committee on Tumor Terminology of the American Society of Clinical Pathologists was held in Chicago, on October 30, 1947. Attending the meeting were: the members of the Committee, Dr. I. H. Perry, chairman, Colonel J. E. Ash, and Dr. W. M. Simpson; and Dr. E. E. Osgood, chairman of the ASCP Committee on Terminology in Hematology; Dr. Bowman Crowell and Dr. Charles F. Branch of the American College of Surgeons; and Dr. George Halpern of the American Medical Association, who sat with the Committee as advisors. Dr. Crowell, Dr. Branch, Dr. Halpern and Colonel Ash gave the historical background of past efforts to promote unity and clarity in tumor terminology.

The Committee accepted the invitation of Dr. Halpern to collaborate with the American Medical Association's Committee on Classification of Tumors.

The Committee agreed to ask other organizations concerned with tumor terminology each to appoint a representative to a Consultative Panel on Tumor Terminology sponsored by the American Society of Clinical Pathologists. Letters will be sent to the following organizations: American Association of Pathologists and Bacteriologists, American College of Surgeons, American Medical Association, International Cancer Research Commission, National Research Council, U. S. Army Medical Corps, U. S. Navy Medical Corps, U. S. Public Health Service and Veterans Administration.

National medical associations sponsoring tumor registries will be asked each to appoint a representative to advise with the American Society of Clinical Pathologist's Committee on Tumor Terminology regarding the coding and listing of the tumors in their specialty. These organizations and the registries they sponsor are: American Academy of Dermatology and Syphilology, sponsoring the Dermal Pathology Registry; American Academy of Ophthalmology and Otolaryngology, sponsoring the Registry of Ophthalmic Tumors; American Academy of Orthopedic Surgeons and American Board of Orthopedic Surgery, sponsoring the Registry of Orthopedic Pathology; American Association of Pathologists and Bacteriologists, sponsoring the Lymphatic Tumor Registry; American Gynecological Society, sponsoring the Registry of Ovarian Tumors; American Neurological Association; American Psychiatric Association sponsoring the Registry of Neuropathology; American Society of Clinical Pathologist's Committee on Terminology in Hematology; American Urological Association, sponsoring the Registries of Genito-urinary Tumors; and the Association for Study of Internal Secretions.

The Committee agreed that the base line from which work should begin is a study of Standard Nomenclature of Diseases and The International List of Causes of Disease and Death; that there should be a code of neoplasia stating for each category of tumors (1) the primary site, (2) the cell derivation and (3) the behavior; that an alphabetical list of tumors should be drawn up designating the appropriate coding of the tumor; that this preliminary

work should be begun as soon as funds for a secretary are available. Colonel Ash offered access to the facilities of the American Registry of Pathologists in the Army Institute of Pathology for this work. The offer was gratefully accepted.

When the code and the alphabetical list are prepared they will be circulated to representatives of the medical specialties for criticism, following which a conference of the Consultative Panel will be held. If the Panel agrees on a terminology, the recommended terminology will be referred back to the organizations represented on the Consultative Panel and the national organizations of medical specialties represented, including the American Society of Clinical Pathologists. If a substantial endorsement of the recommended terminology is obtained, a sustained effort will be made to place it into general use as the approved terminology.

The Committee approved that the Chairman ask for a grant for this work from the U. S. Public Health Service or other organizations whose interest and resources would enable them to sponsor this work.

November 17, 1947

DR. I. H. PERRY, Chairman
Committee on Tumor Terminology
American Society of Clinical Pathologists

BLOOD BANK INSTITUTE

A Blood Bank Institute, sponsored by The William Buchanan Blood Center of Baylor Hospital, was held November 17, 18 and 19, 1947, at the Baker Hotel in Dallas, Texas. The Institute was attended by 125 persons. As a result of the Institute, the American Association of Blood Banks was formed. The objectives of this Association are:

1. To promote and foster the exchange of ideas and materials and the dissemination of information relating to Blood Banking and its technical methodology by education, publicity and research;
2. To foster and plan for cooperation in times of disasters;
3. To function as a clearing house on questions relating to the training of personnel common to such institutions;
4. To keep currently aware of and encourage high standards of service;
5. To promote and foster and aid and encourage the extension of similar services throughout the United States and its territories.

The following were elected as officers of the Association: Dr. G. Albin Matson, Salt Lake City, president; Dr. Leon E. Mermod, Honolulu, vice-president; Miss Marjorie Saunders, Dallas, secretary; Mr. W. Quinn Jordan, Phoenix, Arizona, treasurer; Dr. John Scudder, New York City, president-elect.

The following were elected members-at-large: Dr. Julius Davenport, Jr., New Orleans; Dr. Joseph M. Hill, Dallas; Dr. John Elliott, Miami, Florida; and Dr. Marion Rymer, Denver.

POSTGRADUATE COURSE IN HEMATOLOGY AND BLOOD DISORDERS

A postgraduate course in hematology and blood disorders will be held February 16 through 19, inclusive, 1948, at The Medical Center of the University of California, in San Francisco. Inquiries should be directed to Dr. Stacy R. Mettier, Head of Postgraduate Instruction, Office of Medical Extension, University of California Medical School, San Francisco 22, California.

CORRECTION

Dr. William P. Belk and Dr. F. William Sunderman wish to make the following correction in their article, A Survey of the Accuracy of Chemical Analyses in Clinical Laboratories, which appeared in the November 1947 issue of the Journal, page 853. The errors are believed to have occurred when the raw data were sent to Dr. Charles P. Winsor for statistical analysis. The corrections follow:

TABLE I

	SUBSTANCE TESTED	SATISFACTORY LIMITS OF RESULTS PER 100 ML.	NUMBER SATISFACTORY	NUMBER UNSATISFACTORY	GROSS ERROR
<i>September Analyses</i> (Fifth line)	Sodium chloride	456 \pm 15 mg.	17	24	5
<i>October Analyses</i> (Fifth line)	Sodium chloride	642 \pm 15 mg.	17	20	8
(Sixth line)	Sodium chloride	442 \pm 15 mg.	20	19	5

DEVELOPMENT OF A SINGLE STANDARD SLIDE TEST FOR SYPHILIS*

B. S. KLINE, M.D.

From the Laboratory Department, Mt. Sinai Hospital, Cleveland, Ohio

Evidence is already at hand, that an optimal mixture of essentially chemically pure cardiolipin, isolated by Pangborn²⁴ in 1941 from beef heart, and purified lecithin from the same source gives results of maximum sensitivity and of much greater specificity in tests for syphilitic reagin than do the antigen extracts used in the Eagle, Hinton, Kahn, Kline and Mazzini tests.^{12, 25}

Because of its superior quality, cardiolipin-lecithin antigen should soon replace the antigens now used in tests for syphilis. Furthermore, although not completely specific for syphilitic reagin,¹² cardiolipin-lecithin antigen may well serve as the base for the development of a single standard test for syphilis, worthy of universal adoption. Such a test developed through the cooperative effort of interested serologists and possessing features agreed on by them as optimal, should not be known by any person's name, but should be designated "the standard test for syphilis". This will eliminate the present confusion in the serodiagnosis of syphilis caused by the multiplicity of names, techniques, results and their interpretation.

A standard test for syphilis should have the advantages of maximum specificity, maximum sensitivity, maximum uniformity of results, maximum simplicity and maximum rapidity.

Until now, all blood and spinal fluid tests for syphilis have lacked maximum specificity due in great part to the fact that the antigens employed have been extracts of heart powder and have contained not only the active fractions, but also a variable amount of adventitious material responsible for nonspecific reactions.¹³ Accepting an optimal mixture of essentially chemically pure cardiolipin and lecithin as the standard antigen, it remains to determine the best way to use this reagent in the preparation of the antigen emulsion and the best technique to employ the emulsion in testing blood and spinal fluid for syphilitic reagin.

Before proceeding to a consideration of optimal antigen emulsion preparation, it should be decided what type of reaction should be used in the standard test for syphilis. The complement-fixation reaction requires five ingredients and many hours for its completion. Tests employing it are no longer adequate for modern medical practice in hospitals, particularly in blood donor work and in patients requiring prompt surgical treatment. The flocculation reaction requires two ingredients only and tests employing it can be completed rapidly. Since certain rapid flocculation tests have been found to give results as good or better than the complement-fixation tests,^{5, 32} the direct detection of syphilitic reagin by flocculation of microscopically visible antigen particles is the method of choice.

* Received for publication, November 3, 1947.

The tube flocculation test technic has much to recommend it, especially when it includes the use of concentrated emulsion, optimal ratio of emulsion to serum and acceleration of the reaction by shaking, as in the Kahn test,¹⁰ and also when large and fairly stable antigen particles are used, as in Müller²³ and Kline¹⁴ ball tests, with acceleration by centrifuging and with results which may accurately and easily be read grossly as negative, weakly positive, positive and strongly positive.

The most recently developed flocculation test technic employs concentrated emulsion, optimal ratio of emulsion to serum and mixture of the ingredients on an open glass slide in wax-ringed chambers by rapid rotation. The slide tests are easier to perform and to read accurately and are more economical of time, materials and space than are the tube tests. The slide test technic, which offers the most advantages; is therefore best suited for use in a standard test for syphilis.

A. STANDARD ANTIGEN

As stated above, an optimal mixture of essentially chemically pure cardiolipin and lecithin can serve well as the antigen for a standard test for syphilis. Five slide tests for syphilis with cardiolipin-lecithin antigen already have been reported (Brown,¹ Rein and Bossak,²⁵ Kline,¹² Harris, Rosenberg and Riedel,⁶ and Kahn¹¹). In three of the tests, a ratio of 1 cardiolipin to 9 to 10 lecithin is employed (1C:9 to 10L), in the fourth a ratio of 1C:6.5L and in the fifth the ratio 1C:25L is used. In one of the slide tests,¹² each cc. of chemically standardized cardiolipin-lecithin antigen contains 2 mg. cardiolipin and that amount of lecithin (usually 18 to 20 mg.), which on serologic standardization, determined by tests of nonsyphilitic serums at 1 to 2 C. and of low-titered syphilitic serums at room temperature, gives results of maximum specificity and maximum sensitivity. Further studies should establish the optimal quantity and proportion of cardiolipin and of lecithin to use in a standard slide test for syphilis.

B. STANDARD ANTIGEN EMULSION

Given an antigen for syphilis, standardized chemically for purity and serologically for maximum specificity and sensitivity, it remains to find the best method of employing it in the preparation of an emulsion with which to test blood and spinal fluid for syphilitic reagin. This will require study to determine the reagents to use with the antigen: cholesterol or other coarsener, sodium chloride or other electrolyte, and water, alcohol or other solvents. Furthermore, it will require study to establish the proper method of mixture of the ingredients to obtain stable antigen particles of optimal size and optimal shape for flocculation. Data relating to these factors and to the mechanisms of the flocculation reaction have been reported by Kahn,¹⁰ Kline,¹⁵ Klopstock,¹⁸ Eagle,⁴ Harris, Rosenberg and Riedel.⁶

Reagents of the Antigen Emulsion

1. *Antigen.* The quantity of antigen as well as its quality are of importance in the preparation of an optimal emulsion. A study has shown that maximum

sensitivity is obtained when one full unit is used and that more than this amount results in lessened sensitivity.¹⁶ Use of less than one unit of antigen results in emulsions with incompletely dispersed particles.

2. *Cholesterol* or other coarsener. This reagent should be of highest purity, ash-free and completely soluble in absolute ethyl alcohol to at least 1 per cent at room temperature. Browning, Cruickshank and M'Kenzie,² in 1910, reported that, whereas Wassermann tests with crude alcoholic extract antigens were more sensitive than those with the lecithin fraction of the extracts, the addition of cholesterol to the lecithin solution rendered it at least as sensitive as the crude extract. In 1918, Sachs and Georgi²⁷ introduced the use of cholesterol to increase the sensitivity of the antigen of their flocculation test. Recently, Kahn¹¹ reported the use of gum mastic and of cholesterol with cardiolipin and lecithin in the preparation of the antigen emulsion for his test.

A unique antigen emulsion is that for the Hecht⁷ colored ball test composed of elongated globular sudan-stained lipid antigen particles of variable size. No cholesterol or coarsener other than the dye is employed.

Antigen emulsions made by mixing salt solution with antigen extract or cardiolipin-lecithin solution contain numerous globular particles, varying in size from barely visible or invisible through the microscope to 15 microns in diameter, depending principally upon the quantities of ingredients used. Such non-cholesterolized emulsions are insufficiently sensitive for the detection of small quantities of syphilitic reagin.

There is evidence¹⁴ that cholesterol or like substance increases the sensitivity of the emulsion by changing the antigen particles from globular form and variable size to larger needle or platelike units with flat surfaces optimal for flocculation.

Emulsions generally employed in tests for syphilis are made by mixing salt solution with cholesterolized antigen solution. The resultant aggregates are composed of antigen lipid, cholesterol and other adventitious substances distributed more or less uniformly throughout the particles. The size and the shape of these particles are determined especially by the quantitative relationship of lipid and water, by the temperature of the ingredients and by the speed at which the mixture is made. Antigen emulsions for complement-fixation tests are ordinarily made by mixing from 20 to 100 parts of saline with 1 part cholesterolized antigen extract. Some are water clear. The resultant antigen units are invisible through the microscope and the results of their mixture with syphilitic serum or spinal fluid are usually also invisible and require the use of a hemolytic system for their demonstration. In contrast to such emulsions, those for the flocculation tests are much more concentrated and the antigen particles much larger so that when acted upon by syphilitic reagin, their flocculation is directly observable. A study of flocculation test emulsions made by mixing saline with cholesterolized antigen¹⁴ has shown that in emulsions chemically identical, the particles may vary from less than one micron in diameter, as in the Kahn antigen dilution, and when cholesterolized Kline antigen and abundant salt solution are quickly mixed at room temperature, to large needle-like particles 60 microns or more in length, as in the Müller antigen emulsion, and when cold cholesterol-

olized Kline antigen (at 3 C.) is slowly mixed with very little cold salt solution.¹⁴ The sensitivity of such emulsions, depending in considerable part upon the size and shape of the antigen particles is, therefore, unavoidably variable, and satisfactory results are obtained only by observing the strictest precautions relating to the quantity and temperature of the ingredients, to the speed of the mixture and to the size of the mixing vials. Furthermore, because of secondary changes such emulsions are frequently unstable and unsatisfactory for use several hours after preparation.

In contrast to such emulsions, those for the Kline and for the Boerner and Lukens tests are prepared by first precipitating the cholesterol (from alcoholic solution) in water and subsequently coating the crystals with antigen. Cholesterol plates thus precipitated from alcoholic solution vary comparatively little in size and shape and are stable. Upon these facts depends the greater uniformity in sensitivity of these emulsions. Furthermore, such antigen particles averaging about $5 \times 3 \times 1$ microns are flat, platelike structures, optimal for flocculation, and retain their antigenic power unchanged for at least forty-eight hours.

In addition to cholesterol and gum mastic, balsam of Tolu, sitosterol, benzoin and certain other substances forming emulsions or suspensions on mixture with water, have been used to increase the sensitivity of antigen emulsions in tests for syphilitic reagin.¹⁸

It should be added that to produce large antigen particles and thus secure great sensitivity, certain tests^{6, 23} employ a mixture of a minimal quantity of saline with the antigen followed by a second dilution with saline. The particles thus formed are much larger than when the same quantity of antigen is mixed once only with the same total quantity of saline.

Another method of increasing the sensitivity of an antigen emulsion consists in centrifuging the antigen emulsion, discarding the supernatant and suspending the sediment of large antigen-coated cholesterol crystals in a proper quantity of salt solution.

Further studies of particles of different size and shape and possibly of color containing or capable of holding antigen on their surfaces may reveal those best for flocculation.

3. *Electrolytes.* The sodium chloride used in preparing the emulsion should be of reagent or C.P. quality. An antigen emulsion containing salt gives much more sensitive results than a similar one without it. It is believed that the addition of salt to the emulsion increases its sensitivity by depressing the electro-negative surface charge of the antigen particles and possibly by binding some of the water of the emulsion (electrical neutralization and dehydration effects).¹⁵

In some flocculation test emulsions, as much as 10 per cent sodium chloride solution is employed. In some, ammonium sulfate is used, and in still others calcium chloride is added because of its greater electrolytic activity.¹⁸ In general, however, physiologic salt solution is employed and is probably optimal.

As pointed out by Witebsky,³³ it is possible to flocculate antigen particles by calcium chloride alone, and Kahn¹⁰ and Hernandez⁸ reported false-positive flocculation reactions when a small quantity of hydrochloric acid was added in

the preparation of the Kahn antigen dilution. In contrast to the action of positive ions of acids and of salts, especially divalent ones, Kahn¹⁰ reported false-negative reactions when a small quantity of alkali was added to the antigen dilution.

4. *Water.* Good serologic technic requires that the water employed as diluent be distilled water nearly free of electrolytes, especially of positive ions of acids and of divalent salts (*e.g.*, soluble calcium salts). There is some evidence^{6, 15, 23} that distilled water and salt solution of pH 6 to 7 are optimal for the antigen emulsion.

A certain minimal amount of water is necessary to disperse completely the antigen particles in an antigen emulsion. With less than this amount, as in Kahn antigen dilution, the particles adhere to one another in clumps that resemble those of a positive reaction. When such an emulsion is added to a sufficient quantity of a negative or a positive serum, the antigen particles are completely dispersed by the water present. If the serum contains syphilitic reagin, the particles are altered and flocculation occurs and the resultant clumps resemble those of the original antigen dilution with incompletely dispersed particles. If no reagin is present the particles remain discretely dispersed.

5. *Buffers.* To counteract acid and other positive ions in water, salt and other solutions used in preparing the antigen emulsion, certain tests employ a small amount of alkali^{22, 23} and others^{6, 19} use to better advantage, Sørensen's²³ phosphate, McIlvaine's²¹ citric acid and phosphate or Clark and Lubs'³ phthalate buffers of pH 6 to 7.4.

6. *Alcohol.* For ease in accurately measuring antigen and cholesterol, these ingredients are dissolved in ethyl alcohol. As yet, little attention has been paid to the standardization of this solvent.

The optimal amount of alcohol that the antigen emulsion should contain has not yet been determined. It is possible that it may be none at all. A thoroughly satisfactory emulsion without alcohol has been obtained by centrifuging an emulsion as ordinarily prepared, decanting all the fluid and suspending the particles of the sediment in salt solution.¹⁵

7. *Glycerol.* McGlumphy²⁰ and later Hinton⁹ reported that glycerol could be used with advantage in the flocculation test. When added to antigen it increases its flocculating capacity and adds clarity to the medium so that the reading of results is facilitated.

C. STANDARD TEST CONDITIONS

Given an antigen for syphilis standardized chemically for purity and an antigen emulsion standardized serologically for maximum specificity and maximum sensitivity, the final step in the development of a standard test for syphilis is the determination of the best technic to employ in testing blood and spinal fluid for syphilitic reagin. The test should have the advantages of maximum simplicity and maximum rapidity. As concluded above, the flocculation reaction is the method of choice and the slide test is the technic best suited for use in a standard test for syphilis.

1. *Serum.* Tests with serum (unheated) as obtained after the blood has

clotted, require many precautions to insure uniformity of results.¹⁵ Serum heated at 56 C. from fifteen to thirty minutes and serum heated at 60 to 63 C. from three to five minutes^{17, 26, 30, 31} have been found optimal in the slide tests, as in the other tests, for syphilis.

2. *Quantity of serum.* In the five reported slide tests employing cardiolipin lecithin antigen 0.05 cc. is the quantity of serum used.

3. *Quantity of antigen emulsion.* The optimal quantity of antigen emulsion to add to 0.05 cc. serum should be as small as possible in order to dilute the reagin as little as possible, and yet should be sufficient to contain an optimal number of antigen particles for the test. A ratio of 1 part emulsion to about 7 parts serum has been found satisfactory in the five slide tests.^{1, 6, 11, 12, 25}

4. *Mixing chambers.* The optimal diameter of the paraffin-ringed chambers for mixing 0.05 cc. serum and one small drop of emulsion is about 14 mm.¹⁵

5. *Acceleration of the reaction.* Optimal acceleration of the reaction is obtained by hand rotation of the slide in a circle about three-fourths inch in diameter for four minutes,¹⁵ at the rate of about 180 rotations per minute. Machine rotation must be set by comparison of results with those of standard hand rotation.

(Slide tests should be performed in a place free of drafts to prevent non-specific aggregation of small clumps into larger ones. In a hot dry climate a box lid similar to the slide holder and containing a moistened blotter should be inverted over the holder during the four-minute rotation to prevent completely any evaporation.)

6. *Reading of results.* Optimal reading of results is made through the microscope at a magnification of about 100 times¹⁵ and is reported in terms of pluscs according to the degree of clumping and size of the clumps.

7. *Zone reactions.* Occasionally, there is atypical clumping with very strongly positive serums. Such irregular feathery clumping is not at all like the uniformly distributed small clumps of doubtful reactions with which they could be confused. It is advisable to dilute such serums with saline or negative serum, using one part of the serum in question and 1, 3 and 15 parts of saline or negative serum. The results with one or more of the diluted mixtures will be typical of the strongly positive reaction.

8. *Titer and reagin units.* The titer of a syphilitic serum is the highest dilution of it still giving a 2 plus or stronger reaction. The number of reagin units in a syphilitic serum is the same as the dilution at which the last positive reaction is 2 plus.

9. *Spinal fluid.* Because syphilitic spinal fluid ordinarily contains much less reagin than does serum and is a much less viscid fluid, some modification of the technic for blood will be required in the development of a standard slide test of spinal fluid.

SUMMARY

A standard test for syphilitic reagin should possess the advantages of maximum specificity, maximum sensitivity, maximum uniformity of results, maximum simplicity and maximum rapidity.

An optimal mixture of essentially chemically pure cardiolipin obtained from beef heart and purified lecithin from the same source has been found in tests for syphilis to give results of maximum sensitivity and of much greater specificity than obtainable with present day antigen extracts; accordingly, it may be used as a base for the development of a single standard test for syphilis.

Studies to determine the best method of using cardiolipin lecithin antigen in the preparation of the antigen emulsion for a standard test have been outlined.

The flocculation reaction requiring two ingredients and little time is much better suited for use in a standard test for syphilis than is the complement-fixation reaction requiring five ingredients and much time.

The slide flocculation test technic offers more advantages for a standard test for syphilis than does the tube flocculation technic.

Acknowledgments. I wish to thank Dr. Walter M. Simpson and Mrs. H. Suessenguth, M.T. (ASCP), for helpful suggestions relating to the manuscript.

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CARDIOLIPIN ANTIGENS IN SEROLOGIC TESTS FOR SYPHILIS*

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The isolation of the phospholipin, cardiolipin, from beef heart muscle by Pangborn⁵ in 1941, and its use with purified lecithin as an antigen in the serodiagnosis of syphilis has been followed by several reports^{1, 2, 3, 6, 7} of its adaptability to the various types of present-day serologic methods. Our investigation began soon after Pangborn's reports. Preliminary methods were studied using cardiolipin antigens furnished by Pangborn.

During the past eighteen months, we have employed one or more cardiolipin antigens in tests run parallel with the standard Mazzini⁴ microflocculation test. Cardiolipin and lecithin solutions for these studies were supplied through the courtesy of Dr. Pangborn, the Lederle Company, the Eli Lilly Company, and by Mr. Ad Harris of the Venereal Disease Research Laboratory. The Mazzini antigen was supplied by the author serologist. The various cardiolipin antigens are designated by letter. Those received from the same source, but on different dates and from different batches, are given separate letter designations.

The blood serums studied were received from the following sources: (1) four regional hospitals, (2) the private practice of local physicians, (3) a local U. S. Public Health Service Venereal Disease Clinic and (4) local industrial corporations.

METHOD

The V.D.R.L. (Venereal Disease Research Laboratory) microflocculation test described by Harris² was used exclusively with the cardiolipin antigens in this study. The following deviations from the described technique, which, we believe, do not significantly alter the results, were observed: (1) Serums were inactivated at 56 C. for fifteen minutes rather than for thirty minutes; (2) Slides were of the Kline concave type rather than of the flat type; (3) In a few series, for reasons of experiment, the cardiolipin-lecithin ratio was changed from the prescribed 1:9. These deviations were used for purposes of expediency and are not suggested as modifications.

Cardiolipin, lecithin and cholesterol mixtures were prepared in 10 cc. volumes from separate solutions of cardiolipin and lecithin obtained from the sources mentioned and also from previously prepared mixtures from the Venereal Disease Research Laboratory.

* Presented at the Twenty-Sixth Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 29, 1947. Received for publication, November 17, 1947.

TABLE 1
ANALYSIS OF 24,085 SERUMS BY CARDIOLIPIN AND MAZZINI TESTS

	NUMBER OF SPECIMENS	PERCENTAGE
Complete agreement		
Cardiolipin and Mazzini negative.....	21,590	89.64
Cardiolipin and Mazzini positive (2+ to 4+).....	1616	6.71
Cardiolipin and Mazzini doubtful (\pm to 1+).....	161	0.67
Total.....	23,367	97.02
Partial agreement		
Cardiolipin positive (2+ to 4+), Mazzini doubtful (\pm to 1+)	325	1.35
Mazzini positive (2+ to 4+), Cardiolipin doubtful (\pm to 1+)	34	0.14
Cardiolipin doubtful (\pm to 1+), Mazzini negative.....	231	0.96
Mazzini doubtful (\pm to 1+), Cardiolipin negative.....	26	0.11
Total.....	616	2.56
Complete disagreement		
Cardiolipin positive (2+ to 4+), Mazzini negative.....	80	0.33
Mazzini positive (2+ to 4+), Cardiolipin negative.....	22	0.09
Total.....	102	0.42

TABLE 2
ANALYSIS OF COMPLETE DISAGREEMENT IN 102 CASES

	NUMBER OF SPECIMENS	PERCENTAGE OF DISAGREEMENTS	PERCENTAGE OF TOTAL
Cardiolipin falsely positive.....	8	7.84	0.03
Mazzini falsely positive.....	13	12.75	0.05
Cardiolipin truly positive.....	62	60.78	0.25
Mazzini truly positive.....	7	6.86	0.02
Cardiolipin positive, diagnosis undetermined.....	10	9.81	0.04
Mazzini positive, diagnosis undetermined.....	2	1.96	0.009

ANALYSIS OF DOUBTFUL AND NEGATIVE DISAGREEMENTS IN 257 CASES

Cardiolipin falsely doubtful.....	31	12.06	0.128
Mazzini falsely doubtful.....	11	4.28	0.045
Cardiolipin truly doubtful.....	125	48.64	0.516
Mazzini truly doubtful.....	6	2.34	0.024
Cardiolipin doubtful, diagnosis undetermined.....	75	29.18	0.311
Mazzini doubtful, diagnosis undetermined.....	9	3.50	0.037

TABLE 3
COMPARISON OF CARDIOLIPIN ANTIGENS IN TESTS

	NUMBER OF SERUMS	PERCENTAGE
Antigens A 1:8 and B 1:8, 5039 Serums		
Complete agreement		
A and B negative.....	4489	89.09
A and B positive.....	417	8.27
A and B doubtful.....	59	1.17
	<hr/> 4965	<hr/> 98.53
Partial agreement		
A positive, B doubtful.....	39	0.77
B positive, A doubtful.....	15	0.30
A doubtful, B negative.....	11	0.22
B doubtful, A negative.....	6	0.12
	<hr/> 71	<hr/> 1.41
Complete disagreement		
A positive, B negative.....	3	0.06
B positive, A negative.....	0	—
	<hr/> 3	<hr/> 0.06
Analysis of Complete Disagreements		
A truly positive	3	
Antigens B and E, 1264 Serums		
Complete agreement		
B and E negative.....	1084	85.76
B and E positive.....	50	3.96
B and E doubtful.....	6	0.47
	<hr/> 1140	<hr/> 90.19
Partial agreement		
B positive, E doubtful.....	0	
E positive, B doubtful.....	31	2.45
B doubtful, E negative.....	2	0.16
E doubtful, B negative.....	89	7.04
	<hr/> 122	<hr/> 9.65
Complete disagreement		
B positive, E negative.....	0	
E positive, B negative.....	2	0.16
	<hr/> 2	<hr/> 0.16

TABLE 3—Continued

	NUMBER OF SERUMS	PERCENTAGE
Analysis of Complete Disagreements		
E truly positive.....	1	
E falsely positive.....	1	
Antigens D and F, 1095 Serums		
Complete agreement		
D and F negative.....	991	90.50
D and F positive.....	66	6.03
D and F doubtful.....	27	1.56
	<hr/> 1084	<hr/> 98.09
Partial agreement		
D positive, F doubtful.....	0	
F positive, D doubtful.....	9	0.83
D doubtful, F negative.....	0	
F doubtful, D negative.....	2	0.18
	<hr/> 11	<hr/> 1.01
Complete disagreement		
D positive, F negative.....	0	
F positive, D negative.....	0	
Antigens A 1:9 and D 1:9578 Serums		
Complete agreement		
A and D negative.....	531	91.87
A and D positive.....	35	6.06
A and D doubtful.....	8	1.37
	<hr/> 574	<hr/> 99.30
Partial agreement		
A positive, D doubtful.....	2	0.35
D positive, A doubtful.....	0	
A doubtful, D negative.....	2	0.35
D doubtful, A negative.....	0	
	<hr/> 4	<hr/> 0.70
Complete disagreement		
A positive, D negative.....	0	
D positive, A negative.....	0	

RESULTS

The results of these studies are reported in three groups.

I. Parallel comparative tests were made on routine serums using cardiolipin antigen and Mazzini antigens.

The analysis of this work is presented in Table 1. The cardiolipin tests re-

ported include a composite of all the cardiolipin and lecithin antigens studied. Complete agreement was observed in 97.02 per cent and complete or partial agreement in 99.58 per cent of the 24,085 serums tested. The analysis of the complete disagreement in 102 cases (0.42 per cent of the entire series) and the analysis of the doubtful and negative disagreement in 257 cases is presented in Table 2. From the clinical history of the cases in which we were able to obtain information, 8 of the cardiolipin results were falsely positive as compared with 13 falsely positive Mazzini reactions. However, 62 cardiolipin reactions were truly positive as compared to only 7 of the Mazzini tests. In 12 cases, clinical information was not obtainable. In the doubtful and negative groups, the cardiolipin test gave falsely doubtful results in about three times as many cases as did the Mazzini test, but truly doubtful cases were picked up about twenty times more frequently by the cardiolipin than by the Mazzini test. These results emphasize the high degree of sensitivity of the cardiolipin antigen,

TABLE 4
TITRATIONS OF ANTIGENS

POSITIVE SERUM POOL	3-12-47						4-2-47						9-4-47								
	A	E	F	G	H	Mazzini	A	E	F	G	H	Mazzini	A	B	C	D	E	F	G	H	Mazzini
1:4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
1:8	4	4	4	4	4	2	4	4	4	4	4	2	3	3	2	4	4	4	4	3	3
1:16	2	3	3	2	3	±	3	4	4	3	4	1	2	2	1	2	2	2	2	1	1
1:32	±	±	±	—	1	—	±	1	1	1	1	—	1	1	±	—	±	±	±	±	—
1:48	—	—	—	—	—	—	—	±	—	—	—	—	—	—	—	—	—	—	—	—	—

and this is all the more striking since it is being compared to one of the more sensitive tests using a lipoidal antigen.

II. Group II includes parallel tests using two different cardiolipin antigens on routine serums.

In Table 3 there is a comparison of 5039 serums in which, as an experiment, the cardiolipin and lecithin were used in a ratio of 1:8 rather than 1:9, in antigens A and B. A high percentage of complete and partial agreement was obtained. In the .06 per cent of complete disagreement, antigen A was truly positive in three cases and antigen B was falsely negative. Antigens B and E were compared in tests of 1264 serums. Again a high degree of correlation was found, although the percentage of partial agreement was rather high. Antigen E was observed to be more sensitive than antigen B. The results with antigens D and E in tests of 1095 serums showed a high percentage of agreement with no complete disagreement. Similar results were obtained in testing 578 serums with antigens A and D using cardiolipin and lecithin in a ratio of 1 to 9.

III. The third part of this report concerns the sensitivity levels of different cardiolipin antigens and the Mazzini antigen, using pools of positive serums undiluted and diluted with saline (pH 7.4). The dilutions and some typical results are recorded in Table 4. The uniformity of the cardiolipin antigens is

obvious, and all these antigens exhibit an increased sensitivity as compared to the Mazzini antigen.

COMMENT

Analysis of the results leads to several impressions. It is evident that the Mazzini test and V. D. R. L. microflocculation test using cardiolipin antigen agree in a large percentage of cases. Most of the disagreement encountered is due to a higher sensitivity of the cardiolipin antigens in known syphilitic serums. Some lesser partial disagreement occurs in a slight increase of nonspecific doubtful reactions with cardiolipin. These latter troublesome reactions probably can be reduced without significant loss of true sensitivity by changing the cardiolipin-lecithin ratio. Serologic assay for adjusting the sensitivity level of these antigens should include tests from groups of cases most likely to give these nonspecific doubtful reactions with a view toward adjustment to a level that will avoid the maximum number of them.

The results of comparative tests using different cardiolipin antigens show that their reactivity can be consistently reproduced and that the variations between different lots of antigens are of such slight degree that these mixtures will lend themselves to standardization much better than lipoidal substances. The production on a large scale of antigens giving uniform reactivity is anticipated.

SUMMARY

1. Cardiolipin-lecithin antigen exhibited consistently reproducible levels of specificity and sensitivity.
2. In comparison to the Mazzini test, the cardiolipin-lecithin antigen in the VDRL (Venereal Disease Research Laboratory) test was more sensitive in positive serums and about equally specific in negative serums.

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CARDIOLIPIN BLOOD TESTS IN SYPHILIS*

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Cardiolipin is a complex phosphatidic acid discovered in 1941 by Mary Pangborn¹⁴ of Albany, New York, while she was studying the alcoholic extract of beef heart in search of the single substance which gives this extract its antigenic qualities. Although it was subsequently purified and studied in 1942¹⁵ and 1945,¹⁶ it was not until 1947 that she reported¹⁷ the tentative formula of cardiolipin and its approximate chemical composition. On alkaline hydrolysis it yields oleic and linoleic acids plus a polyester of glycerophosphoric acid and glycerol.

Cardiolipin has not as yet been synthesized, but it is apparently a single chemical substance, reproducible as such. Alone it is not antigenic, requiring lecithin and cholesterol for antigenicity. All three of these substances are, however, quite reproducible, so that presumably, within the limits of the reagin-antigen circle, we are a long stride nearer serologic Utopia. This can be true, however, only if the new antigen stands up under the critical light of clinical trial.

SCOPE OF STUDY

It has been our purpose to study this antigen not only from the laboratory standpoint, but from the clinician's viewpoint. To achieve the former, we contrasted cardiolipin with a "battery" of other tests. To achieve the latter, we employed serums of: (1) patients with clinically known stages of syphilis, treated or untreated; (2) patients with other diseases and states yielding so-called false-positive reactions; and (3) normal healthy individuals. These blood studies were run during a period of approximately one year by two competent serologists in two separate laboratories. The serums were obtained from: (1) a large private hospital with a capacity of more than four hundred; (2) a charity hospital of 185 beds; (3) a charity venereal disease clinic with a large Negro registration; and (4) a group of presumably healthy donors, consisting largely of college students, soldiers, sailors and industrial workers. A total of 24,609 different blood samples (not necessarily consecutive) were examined by at least two, and often six or more, of the following tests: Kline exclusion, Kline cardiolipin exclusion, Kline diagnostic, Kline cardiolipin diagnostic, Kahn standard, Kahn quantitative, Kolmer quantitative Wassermann and Kolmer quantitative Wassermann using cardiolipin (Harris and Portnoy⁶). We also attempted a modified Mazzini test using cardiolipin but abandoned this study. Mr. Mazzini himself is not working on this project since the Harris VDRL (Venereal Disease

* Presented at the Twenty-Sixth Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 29, 1947. Received for publication, October 29, 1947.

Research Laboratory) flocculation test⁷ is similar to the Mazzini procedure. We also ran 124 samples of blood using the new Kahn cardiolipin microfloculation test on heated serums, but were unable to overcome technical difficulties, especially interpretative, and are therefore not reporting these results.

TECHNICAL NOTES

1. *Kline flocculation tests.* The Kline exclusion test was run according to usual strict technic with regular Kline antigen provided by LaMotte Chemical Company of Baltimore. The Kline cardiolipin exclusion test was performed according to the technic suggested by Kline,⁹ but the ratio of lecithin to cardiolipin was varied two-tenths of unity with each group of about one thousand blood samples. The exact number in each group is listed later, with results. It should be emphasized that the lecithin-cardiolipin proportions are calculated by weight. Thus, to obtain a proportion of 10 parts of a lecithin solution, having 34 mg. per cc. to one part cardiolipin solution, having 9.22 mg. per cc., the formula would

be $\frac{9.22 \times 10}{34} = 2.71$ cc. of the lecithin solution to 1 cc. of cardiolipin solution. Any aliquot

preserving the same ratio will be obviously satisfactory, as long as the cholesterol crystals are properly coated. A few trials of different times and speeds of centrifugation readily provide a smoothly dispersed emulsion. The minimum ratio used was 8 parts of lecithin to 1 of cardiolipin; whereas the maximum was 11.4 to 1. The Kline diagnostic test (uncentrifuged) was performed with LaMotte antigen by strict technic. The Kline cardiolipin diagnostic test was initially performed with several different cardiolipin-lecithin ratios, but these offered little additional information over that previously obtained with the exclusion procedure. Therefore, all of the diagnostic tests here reported were run with a proportion of 1 part of cardiolipin to 9.4 of lecithin. In both techniques we were impressed with the stability of cardiolipin, not only in the mixture with lecithin, but even in the emulsion. During the year, we worked with three different lots of cardiolipin. All three lots yielded smooth, uniform emulsions of quite homogeneous dispersability and practically identical appearance from one run to another. Of course, when low ratios of lecithin to cardiolipin were used (below 8 to 1) the emulsion proved increasingly coarse and difficult to read. Such low proportions of lecithin were also too undersensitive to be of any value, and are not here reported. Storage of the mixed cardiolipin and lecithin, in dark glass bottles, for many weeks did not appear to affect its qualities seriously. Separately the solutions keep for years. Refrigeration of cardiolipin, and of lecithin, is, of course, not indicated; any cool dark place is satisfactory. In the emulsion form the cardiolipin-lecithin-cholesterol mixture was usable even after one week in the refrigerator, whereas ordinary Kline emulsion deteriorates in a matter of hours or days. We were surprised to note that one emulsion, kept in the refrigerator more than six months, was still quite antigenic.

2. *Kahn tests.* The Kahn standard and quantitative tests were done by usual strict technic, using Kahn antigen prepared by the Texas State Department of Health Bureau of Laboratories. The Kahn cardiolipin microfloculation test was performed with both the 0.1 per cent and the 0.25 per cent cholesterol as recommended by Kahn.⁸ Our disappointing experience precluded a lengthy series and, therefore, the results are not reported here.

3. *Kolmer Wassermann tests.* The usual 5 dilution Kolmer quantitative test was run with two commercial regular antigens, carefully following the accepted Kolmer technic. In the case of the quantitative Wassermann using cardiolipin, the emulsion was prepared and employed according to the Harris and Portnoy technic⁶ using 5 dilutions of serums with an antigen of 0.03 per cent cardiolipin, 0.05 per cent lecithin and 0.3 per cent cholesterol in ethanol. Here again the same stability and reproducibility were noted. No effort was made to check the Maltaner application¹⁰ of cardiolipin to the Wadsworth and Maltaner quantitative complement-fixation technic. The regular Kolmer antigens require painstaking standardization and titration. Even with meticulous techniques, an occasional lot of

regular antigen will leave much to be desired in specificity or sensitivity, or both. On the other hand, our Wassermann cardiolipin emulsions were quite reproducible and equally stable. Even the emulsion itself was satisfactory for many days after preparation. Absorption with sheep cells was routinely done; no egg albumen was used. All complement was lyophilized; in one laboratory it was obtained commercially and in another laboratory it was supplied by the Texas State Department of Health. A rough estimate of coloration of the serums was made on outstanding specimens. Hemolysin titration was, of course, repeated after over-night incubation, employing both positive and negative controls. Some effort was made to study different concentrations of cholesterol; at one time we used 1/100 of the recommended amount and we were surprised to note that the emulsion, even with such an unusually low cholesterol concentration, yielded some satisfactory results. Since these tests were in the nature of trial runs and did not yield reliable findings, the results are not recorded. Only results obtained with strict Harris and Portnoy technic are reported here.

4. *Other tests.* We were interested in a number of other tests including the Mazzini modification (micro- and macroprecipitation tests of Brown^{2,3}), the VDRL flocculation test of Harris *et al.*,⁷ and the Rein and Bossak micro-flocculation test.¹⁸ These tests, however, were either not run in this series or the results with their use will be reported elsewhere.

One of the more curious phenomena encountered was a surprising variation in the lecithin stability and reproducibility. We had expected possible variation in cardiolipin but not in lecithin; we found exactly the reverse. Kahn, Harris and Pangborn herself also noted this distressing variability in the alcoholic solution of lecithin. Some months ago, however, the method of preparation was revised and we are reliably informed that it is now quite stable and reproducible. Incidentally, the solvent used in both is a mixture of 9 parts of ethanol and 1 part of methanol. The denaturing with wood alcohol apparently is done for legal rather than for scientific purposes.

RESULTS

The *gross* results obtained with increasing proportions of lecithin to cardiolipin in the Kline exclusion (centrifuged emulsion) tests are outlined in Table 1. Later tables record the sensitivity in known luetics and the specificity on our presumed healthy individuals. The 24,381 blood samples tested were not consecutive since, on several occasions, for various reasons, such as breakage of tubes, two Kline tests could not be run; such cases were not included. In an additional group of 228 no Kline exclusion test was run with either antigen.

Although few conclusions are possible from a table of gross results it is obvious that increasing amounts of lecithin definitely raise the sensitivity. We did not observe this, however, at the low level implied by some workers. One investigator, for example, uses less than 7 L to 1 C (7 parts lecithin to 1 part cardiolipin) and claims oversensitivity beyond that concentration. Naturally the technic employed vastly affects the sensitivity; in one procedure a ratio of 26 L to 1 C is said to be optimal. Even Kline's optimal figure of 9.4 L to 1 C seems, from this table, a bit conservative. From these uncorrected figures alone it would appear that a proportion of 10 L to 1 C or even 10.4 L to 1 C would increase the sensitivity without too great a sacrifice of specificity. Since the cardiolipin-lecithin mixture recently marketed by LaMotte Chemical Company has a 10 to 1 proportion sanctioned by Dr. Kline, it would appear that he is now in fundamental agreement.

TABLE 1
RESULTS OF 24,381 DUPLICATE KLINE EXCLUSION TESTS USING REGULAR AND
CARDIOLIPIN ANTIGENS

KLINE EXCLUSION ANTIGEN USED	NEGATIVE (-)	DOUBTFUL ± OR +	POSITIVE 2+, 3+, 4+	TOTAL TESTS
C1 to L 8.0	207	66	41	314
Regular	173	94	47	314
C1 to L 8.2	581	96	29	706
Regular	602	87	17	706
C1 to L 8.4	566	117	209	892
Regular	617	108	167	892
C1 to L 8.6	703	104	112	919
Regular	581	61	277	919
C1 to L 8.8	804	107	186	1097
Regular	692	156	249	1097
C1 to L 9.0	681	34	80	795
Regular	629	68	98	795
C1 to L 9.2	804	87	217	1108
Regular	1023	34	51	1108
C1 to L 9.4	493	44	67	604
Regular	420	81	103	604
C1 to L 9.6	621	158	214	993
Regular	525	177	291	993
C1 to L 9.8	1196	69	238	1503
Regular	1248	107	148	1503
C1 to L 10.0	3852	429	1437	5718
Regular	3713	453	1552	5718
C1 to L 10.2	2856	181	646	3683
Regular	2941	218	524	3683
C1 to L 10.4	861	154	237	1252
Regular	797	182	273	1252
C1 to L 10.6	1228	57	118	1403
Regular	1193	63	147	1403
C1 to L 10.8	545	67	261	873
Regular	607	39	227	873
C1 to L 11.0	691	16	104	811
Regular	708	22	81	811

TABLE 1—*Continued*

KLINE EXCLUSIVE ANTIGEN USED	NEGATIVE (-)	DOUBTFUL ± or +	POSITIVE 2+, 3+, 4+	TOTAL TESTS
C1 to L 11.2.....	582	79	303	964
Regular.....	713	66	185	964
C1 to L 11.4.....	417	53	276	746
Regular.....	560	31	155	746
Total Cardiolipin-lecithin Tests.....	17,688	1918	4775	24,381
Total Kline Exclusion.....	17,742	2047	4592	24,381

In an unclassified group such as this, the separate runs in each proportion frequently had few or many syphilitics, depending on circumstances. But, in general, there is fairly close correlation between the two antigens, especially when the lecithin concentration was between the proportions of 9 L to 1 C and 10 L to 1 C. In the group of 1108 tests, representing the proportion of 9.2 L to 1 C, the regular Kline antigen was unusually rough and troublesome; therefore, the results are probably unusually undersensitive. In view of the uniform increase in sensitivity with rising lecithin concentration, it seems possible that an uncentrifuged (diagnostic) cardiolipin Kline could be performed at "screen" or exclusion level simply by sharply increasing the proportion of lecithin.

More significant than the gross results of Table 1 is the analysis of reports on luetics of various stages, and on nonsyphilitics. Here the Kline exclusion figures reported include those between the ratios of 9.2 L to 1 C and 10.8 L to 1 C only. At the levels represented by these proportions, more than one-half of all our Kline exclusion tests were run. Here again a negative (-) result is so recorded, a ± or plus reading is considered doubtful, and a 2 plus, 3 plus or 4 plus result is positive. The Kahn standard, Kline diagnostic (regular and cardiolipin) and the Kolmer quantitative (regular and cardiolipin) tests were done on about one-quarter of the 17,137 blood specimens represented by the Kline exclusion figures. In the primary group most of the luetics had penile or labial chancres, but darkfield examinations were not always positive due to self treatment, delay in seeking aid and other factors. The tertiary luetics had a wide variety of neurosyphilitic and cardiovascular lesions. Most of the congenital syphilitics were infants and children under 10 years of age. Naturally a number of the clinical diagnoses are open to further study. The usual criteria of negativity and positivity suggested by the author serologist are followed with one exception: a single minimal positive (plus) in the first of the five Kolmer dilutions (10000) is reported as doubtful rather than as positive.

The advent of penicillin and rapid arsenical treatments have made difficult the interpretation of the figures in Table 2. Many of these patients were semi-migratory workers of the war and reconstruction periods, receiving some treatment in California, some in Tennessee, or elsewhere, others were treated in the armed services. Not infrequently there was reversal of seropositivity with penicillin, followed by still later changes.

Again, the ordinary criteria of sensitivity and specificity are not always easy to apply to cardiolipin antigens, as emphasized in the section of case reports.

TABLE 2

RESULTS OF TESTS ON BLOOD OF KNOWN SYPHILITIC STATUS WITH REGULAR AND CARDIOLIPIN ANTIGENS FOR KLINE AND KOLMER ANTIGENS AND REGULAR KAHN ANTIGEN

SYPHILITIC STATUS	NUMBER TESTED WITH KLINE EXCLUSION	RESULTS*	KLINE EXCL. REGULAR	KLINE EXCL. CARDIO-LIPIN	KAHN STANDARD	KLINE DIAG. REGULAR	KLINE DIAG. CARDIO-LIPIN	KOLMER QUANT. REGULAR	KOLMER QUANT. CARDIO-LIPIN
Primary	94	Negative	67	52	61	44	34	59	49
		Doubtful	11	19	5	13	17	3	7
		Positive	16	23	9	15	21	11	18
Secondary	156	Negative	22	27	19	20	14	31	21
		Doubtful	16	9	12	12	23	8	12
		Positive	118	120	73	99	94	66	78
Latent	2861	Negative	86	59	209	151	84	173	91
		Doubtful	124	313	77	92	66	48	57
		Positive	2651	2489	1818	2026	2139	2008	2064
Tertiary	672	Negative	162	94	216	142	107	71	31
		Doubtful	143	61	83	132	43	12	7
		Positive	367	517	235	282	408	539	585
Congenital	72	Negative	11	7	19	6	3	12	8
		Doubtful	6	7	4	12	9	4	3
		Positive	55	58	47	38	43	33	37
Doubtful or unknown	1659	Negative	762	729	1053	714	706	732	698
		Doubtful	855	734	414	572	516	26	47
		Positive	42	196	36	28	93	28	36
Nonsyphilitic (?)	11,623	Negative	11,357	11,488	8704	386	359	363	359
		Doubtful	199	103	61	27	16	2	3
		Positive	67	32	105	19	34	4	7
Totals	17,137	Negative	12,467	12,456	10,281	1463	1307	1441	1257
		Doubtful	1354	1246	656	860	690	103	136
		Positive	3316	3435	2323	2507	2832	2689	2825
Grand Totals			17,137	17,137	13,260	4830	4829	4233	4218

NOTE: Only Kline exclusion cardiolipins with lecithin proportions between 9.2 and 10.8 to 1 cardiolipin are reported here. All Kline diagnostic cardiolipins are at proportion 9.4 L to 1 C.

*Doubtful: \pm , +; Positive: 2+, 3+, 4+.

Thus, all of the tests may be quite positive, with cardiolipin entirely negative, and time may prove the new antigen correct. Barnard, who believed that 35 per cent of persons considered to be syphilitic in the armed forces never had

syphilis in the first place, and Rosahn,¹⁹ who found gross or microscopic evidence of syphilis at necropsy in fewer than one-half of supposed luetics, should be highly interested in this antigen. Or, just as easily, cardiolipin may be positive while all other tests are negative, and the patient will suddenly "remember" he had a chancre and later a rash for which he had been treated years before.

In general terms, however, both the cardiolipin flocculation tests (Kline exclusion and diagnostic) were slightly more sensitive than the regular corresponding antigen in all five forms of syphilis reported here, with few exceptions. Both appeared appreciably more sensitive than the Kahn test with regular antigen, although this comparison is less impressive since the same number of tests was not run with both procedures. No comment on the specificity of the Kline exclusion tests is warranted, since both antigens in these tests are considered useful largely for screening purposes. In a previous study,¹ limited to Kline exclusion tests only, we found somewhat less sensitivity than in the present series. The Kline diagnostic test with cardiolipin, however, appeared to be slightly more specific than its regular antigen counter-part.

A comparison of the Kolmer quantitative complement-fixation test with both antigens suggested a definite but slight superiority in sensitivity with approximately equal specificity. It should be emphasized, however, that the normal or nonsyphilitic group in these tests did not include anyone with a history of malaria or jaundice, or anyone who had other positive tests, so that false-positivity was less likely. In our series, there were 59 anticomplementary results with regular antigen and 34 with cardiolipin. Haziness in the control tube was considered anticomplementary and the test was not reported. Only 29 serums were anticomplementary with both. In regard to highly icteric serums, we noted no very significant difference with the two antigens as long as the reagin level was high. In the very weakly positive serum, however, there was a decided advantage to cardiolipin. On many such tests it was weakly positive when regular antigen yielded the usual false-negative results previously described by the other workers. Bohls, Shaw and co-workers of San Antonio are presently studying this interesting aspect of the icterus problem.

Naturally the problem of the "biologic" nonspecific positive reaction is of much interest with any new antigen or procedure. Table 3 shows the results with several common sources of erroneous positive reports.

While our number of old malaria cases (mostly tertian and a few estivo-autumnal) was small, the increased specificity obtained by Kline in his much larger series is duplicated, but less impressively. Several false-positive reactions did occur in our series, although not with the frequency encountered by Stout.²⁰ The results with other diseases were less clear. Viral pneumonia, for example, is not always an easy diagnosis to make or substantiate, but the increased specificity claimed by others was not shown at all on flocculation tests and only slightly with the complement-fixation procedure in our cases. In pregnancy, upper respiratory infections, infectious mononucleosis and vaccinia, there was slight but definite increased specificity with cardiolipin, especially in the complement-fixation test. It should be emphasized that in each group false-positive reactions did occur. No substantial difference was noted in acute infectious lympho-

cytosis, brucellosis, or Vincent's angina. The small number of cases in these and several of the other groups is hardly conclusive. Blood samples from leprosy patients were not available.

Again, in general terms, cardiolipin is very slightly more specific in several of the common conditions associated with false positivity. Some of the enthusiastic earlier reports in this respect are not here duplicated, however.

TABLE 3
RESULTS OF 180 TESTS ON FALSE-POSITIVES
(Same Test and Criteria for Negative, Doubtful and Positive as in Table 2)

NUM- BER OF CASES	DIAGNOSIS	KLINE EXCL. REGULAR			KLINE EXCL. CARDIOLIPIN			KAHN STANDARD			KLINE DIAG. REGULAR			KLINE DIAG. CARDIOLIPIN			KOLMER QUANT. REGULAR			KOLMER QUANT. CARDIOLIPIN		
		N	D	P	N	D	P	N	D	P	N	D	P	N	D	P	N	D	P	N	D	P
2	Acute infec- tious lym- phocytosis	1	1	0	1	0	1	0	0	2	1	1	0	2	0	0	1	0	1	1	1	0
14	Acute infec- tious mon- ocyctosis	8	3	3	11	1	2	6	6	2	10	0	4	12	0	2	5	5	4	8	4	2
48	Acute upper respiratory infection	36	4	8	38	3	7	41	6	1	37	6	5	40	3	5	35	4	9	41	3	4
31	Malaria tert. early	24	3	4	28	1	2	24	5	2	25	4	2	30	0	1	19	8	4	25	3	3
37	Pregnancy 2d, 3d trim.	21	7	9	17	8	12	20	6	11	24	9	4	22	6	9	30	2	5	34	1	2
11	Puerperium, early	10	0	1	9	1	1	8	0	3	11	0	0	11	0	0	6	3	2	9	0	2
7	Brucellosis	5	0	2	6	0	1	5	2	0	6	1	0	6	0	1	4	2	1	3	2	2
8	Vaccinia (youths)	4	1	3	7	0	1	5	0	3	4	2	2	8	0	0	3	3	2	7	0	1
5	Viral pneu- monia	1	1	3	2	0	3	1	0	4	1	1	3	2	0	3	1	1	3	3	1	1
17	Vincent's angina	9	2	5	8	5	4	14	2	1	8	4	5	7	6	4	12	1	4	10	3	4
180	Totals	119	22	38	127	19	34	124	27	29	127	28	25	140	15	25	116	29	35	141	18	12

Far more interesting to us were several of the individual cases which are included under the heading of "Doubtful" in Table 2 and which bear individual surveying. Such patients cannot be covered in any system of tabulation, yet each illustrates a problem. These patients afford a welcome change from the white, gray and black of serologic evaluation studies, illuminating in full colors all shades of lues, false-positivity and false-negativity. As pointed out by Giordano,⁴ these cases are more of a problem in every day hospital practice than in national evaluations, where they do not often appear. It should be emphasized that each case described represents only one sample of a definite group of cases.

ILLUSTRATIVE CASES

Case 1. Mrs. K. B., 36, white, was delivered of a normal full-term male on October 27, 1946. A routine Kline diagnostic test was 4 plus. Other tests then done showed Kline exclusion 4 plus; Kahn quantitative 160 Kahn units; Kolmer quantitative 44320. The husband, new son and all other children were negative. Several previous serologic tests for this and previous pregnancies had all been negative. The heterophil antibody test also was negative and there had been no malaria or vaccination. Serum stored in the ice box showed no diminution in titer after four and ten weeks. All three tests (Kline exclusion, diagnostic and Kolmer quantitative) with cardiolipin gave entirely negative results. Six months later the only change was a drop in regular Kolmer titer to 32110; the cardiolipin test was still negative and all regular flocculation tests were strongly positive. In September 1947, the results were quite different: regular Kline exclusion 1 plus; Regular Kline diagnostic 0; Kahn quantitative 0 Kahn units; regular Kolmer 10000. All cardiolipin versions continued negative. While it is doubtful that the patient's husband, a physician, would have been much concerned, still the consistently negative cardiolipin results strongly reinforced the obvious diagnosis of false-positive serologic test in the puerperium.

Case 2. Mr. B. Y., age 17, underwent a routine preschool physical examination in August which revealed a Kline diagnostic test of 4 plus. A Kline exclusion test also with regular antigen was 4 plus; the Kahn 344,444 with 160 Kahn units; regular Kolmer 43220. All three cardiolipin tests (Kline exclusion, Kline diagnostic and Kolmer quantitative) were entirely negative. The serologic studies were repeated in two other laboratories with practically identical results. On careful examination and questioning, it developed that several weeks earlier he had been vaccinated for school certification. There being no history or findings at all suggestive of lues, the youth was advised to continue his education without further ado. Developments since then, showing spontaneous return toward seronegativity, have confirmed the wisdom of this advice. Similar cases are included in our table.

Case 3. Mrs. V. M., 29, white, a divorcee, never pregnant, had influenzal pneumonia several months before routine serologic test prior to marriage. Kline exclusion was 3 plus, but she was given a certificate by her physician, and she was married. Later tests showed Kline exclusion 3 plus; Kline diagnostic 4 plus; Kahn 444,444; Kolmer 42100. All three cardiolipin tests were negative. The issue was clouded, however, because her new husband had blood and spinal fluid evidence of seropositivity with all tests and all antigens. In the ensuing recriminations a divorce resulted. A year later her tests became entirely negative without treatment, but all concerned are more than a little uneasy over the future.

These first three cases illustrate the typical situations in which cardiolipin tests are entirely negative in direct conflict with strongly positive regular tests. Cases 1 and 2 represent no problem, but the situation in Case 3 is somewhat confusing. On the other hand, we have those cases in which cardiolipin tests are strongly positive with entirely negative regular tests. Such results may be more satisfactory to the serologist but they are most distressing to the syphilologist. In our experience these were surprisingly plentiful and we present three more cases selected at random.

Case 4. Mr. M. F., 24, white, veteran, appeared as a donor in the blood bank in May 1947, certifying that he had never had syphilis, malaria or jaundice. After blood was collected his Kline exclusion cardiolipin test was found to be 4 plus. Regular Kline exclusion test was negative; diagnostic negative; Kahn 0; Kolmer 00000. The cardiolipin test showed Kline exclusion 4 plus; diagnostic 4 plus; Kolmer 41000. Confronted with this evi-

dence he admitted having had syphilis in the Army, for which he was "successfully treated". His need for funds prompted him to misrepresent the facts in order to enroll as a professional blood donor. Whether or not he is cured, of course, is another problem, and the answer may never be known for, as pointed out by Moore and Shamberg,¹¹ one-half of all acquired syphilitics suffer no apparent damage even without treatment.

Case 5. Mr. R. I., 51, white, aircraft worker, was admitted in January 1947, with precordial pain and a story suggesting coronary disease. Absolutely no history of lues could be elicited. The Kline diagnostic cardiolipin test showed 3 plus and the regular test was negative. Kline exclusion test was then done with negative results and the Kolmer was 00000. With cardiolipin, however, the Kline exclusion was 4 plus; the cardiolipin Kolmer 32200. When cross-examined and shown these reports he admitted having had a chancre and rash twelve years before. He stated, however, that he had been fully treated by a competent syphilologist and pronounced cured after two and one-half years with consistently negative blood and spinal fluid tests since then. On physical and x-ray examinations, a pulsating mass in the arch confirmed the clinical impression of aneurysm of the aorta. It seems quite likely that this patient's lues was either not cured or that a second infection supervened.

Case 6. Mrs. W. D., about 30, white, was admitted to the prenatal clinic in October 1946. Kline diagnostic test was negative; the Kolmer 00000. With cardiolipin the Kline exclusion test was 4 plus; the Kline diagnostic 2 plus; the Kolmer quantitative 42000. In the face of denial of lues, no treatment was administered although not without some misgiving. The regular Kline exclusion test was done twice in the meantime and was negative.

In April 1947, she was admitted for delivery with the following reports: *regular*: Kline exclusion negative, Kline diagnostic 1 plus, Kolmer 00000; *cardiolipin*: Kline exclusion 4 plus, diagnostic 4 plus, Kolmer 32200. After uneventful labor a stillborn syphilitic fetus was delivered. Treatment of the mother was then instituted, although with a rather rueful feeling.

The last three cases, therefore, illustrate one of the more curious problems in dealing with cardiolipin, the persistent seropositivity years after clinical "cure". How shall the patient be handled who is persistently positive to the cardiolipin test and negative to the regular test? Does he have the probability of recurrence? Although two of the last three cited cases showed recurrence or continuity of syphilis, we had far more patients in the group represented by Case 4. Presumably, with cardiolipin there is a real possibility that, serologically, once a syphilitic always a syphilitic.

DISCUSSION

In reviewing the problem and trying to evaluate the status and future role of cardiolipin, several questions at once come to mind.

1. Is cardiolipin technically easier to work with? The answer is a strong "yes". Not only does it keep better in all forms, but it yields a reproducible, smooth flocculation emulsion, and a clear readable complement-fixation procedure with fewer anticomplementary reactions. Even with highly icteric low-titered positive serums there is sufficient complement fixed to yield a weak positive result instead of the common false-negative. The minor but distressing variation in lecithin which we noted has apparently been conquered lately. The stability of cardiolipin is too well proved to require additional comment. Its reproducibility is of the same high order.

2. Does cardiolipin provide higher sensitivity and greater specificity than other antigens? Here the answer is a much softer "yes". The earlier reports suggesting a nearly perfect antigen will bear further study. In our hands cardiolipin yielded false-positives in both flocculation and complement-fixation tests. There was, however, undoubted superiority in specificity in malaria and increased sensitivity in old treated syphilis. The latter finding provides food for much thought, since the clinician who has serologically "cured" his patient now may find him positive again. It is quite possible that some "negative" serums in state or national evaluation studies may not have been negative had cardiolipin been used. Another interesting situation is offered by the premarital serologic tests now required in many states. The positive results in old (presumably cured)luetics may be most embarrassing to them. On the other side of the ledger, as reported by Kline,⁴ this antigen is making it possible for veterans who have had malaria to get marriage certificates, since their serologic tests are so frequently negative with cardiolipin. In several other diseases the performance in regards to specificity was not especially outstanding. It also must be admitted that the slight increase in sensitivity could be met by raising the cholesterol level in the regular Kolmer test, for example. Whether an accompanying decreased specificity would be important or not would have to be further explored. Certainly the increased specificity of cardiolipin is an undoubted advantage at the present time. Yet, we must freely admit that false biologic positive results in other diseases and in normal individuals are possible. Perhaps the answer to the whole problem of the biologic positive lies not so much in the antigen field as in the globulin fractionation of the serum to be tested. Neurath and associates¹² have shown that the first and second globulin fractions together have lower titer than the whole serums inluetics, but a higher titer in false-positives. Certainly this is an encouraging lead in a new direction.

3. What is the present role of cardiolipin? In general the answer would have to be "somewhat disappointing compared to its potentialities". A plethora of new serologic tests has arisen, together with a spate of cardiolipin modifications of old tests. The clinician needs fewer and better, not more tests. He is bewildered by the flood of flocculation tests; the Kline, Eagle, Hinton, Ide, Rein-Bossak, Laughlen, Boerner-Lukens, Sachs Georgi, Brown, Butler, Vernes, Meinicke, Mazzini, VDRL and Kahn tests offer a terrifying complexity of choice. It is no longer informative to say "the Kahn test is positive". Is the Kahn standard, the Kahn presumptive, the Kahn verification, the Kahn quantitative, the Kahn cardiolipin macro-flocculation, or the Kahn cardiolipin microflocculation meant? Admitting that there is no truly specific test for syphilis, and that cardiolipin is still in the antigen-reagin circle of "practical" specificity, surely more benefit can be derived from this discovery than from a gush of confusing new tests. Patenting and licensure alone will not solve this problem.

4. What is the eventual role of cardiolipin? At this stage even the amateur seer would reply "clouded". All syphilologists dream of a Utopia where there are two, or at most three, simple, reliable, rapid world-wide flocculation tests (at screen and diagnostic sensitivity levels, including perhaps a tube test as suggested by Harris and Mahoney⁶) with one single quantitative complement-

fixation test. Suitable short universal names should be devised for such tests, and preferably these titles should be noneponymic. All of the tests would be equally performed, entirely reproducible and entirely evaluable, everywhere. Even positive artificial standards, such as Brown's Super-Cel and glass standards,³ would be prescribed. Until now the syphilologists' dream is just that. Cardiolipin makes this dream a near reality if (and a big if it is) some group or power can achieve at least nation-wide standardization. Close and unselfish cooperation between many elements would be prerequisite to success. Such action would not impede further research or development, but rather would capitalize on cardiolipin's chief twin advantages: stability and reproducibility. To achieve this dream would be a glorious accomplishment for any organization; to do less would be to deny cardiolipin its maximum usefulness.

SUMMARY

A total of 24,609 blood samples was subjected to 81,391 tests over a period of approximately one year.

Analysis of results using regular and cardiolipin antigens in duplicate flocculation and complement-fixation procedures indicate generally superior specificity and generally very slightly increased sensitivity with cardiolipin.

False-positives did occur with the new antigen, and in a variety of diseases and states.

Problems connected with sharply divergent results in old treated syphilis and in biologic false-positivity are delineated by case reports.

The chief advantages to cardiolipin are stability and reproducibility. At present these advantages are only partly utilized.

To develop the latent benefit now locked in this antigen, sponsored universal tests based on cardiolipin are urged.

Acknowledgments. We are greatly indebted to Dr. J. Franklin Campbell and staff of the Fort Worth Venereal Disease Clinic for their generous cooperation, and Dr. H. D. Piersma and the Lederle Laboratories for liberal supplies of antigens as well as for valued information.

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CLINICAL AND SEROLOGIC EVALUATION OF 27,103 CONSECUTIVE SLIDE TESTS WITH CARDIOLIPIN- LECITHIN ANTIGEN AND KLINE ANTIGEN*

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Since the introduction by Pangborn,⁸ in 1941, of eardiolipin-lecithin antigen in the serodiagnosis of syphilis, clinicians and serologists everywhere have been interested in the results obtainable with it in flocculation and complement-fixation tests. Without exception, the reports^{1-7, 9} in the literature to date have been favorable, and a few,^{5, 6} have clearly shown that proper mixtures of essentially chemically pure cardiolipin and lecithin from beef heart are very much superior to the antigen extracts, from the same source, that are in general use today.

In a recent comparative study,⁵ optimal cardiolipin-lecithin antigen, in the microscopic slide precipitation test for syphilis, gave results of maximum sensitivity and of much greater specificity than did Eagle, Hinton, Kahn, Kline and Mazzini antigens.

The proportions and quantity of cardiolipin and lecithin which give results of maximum specificity and maximum sensitivity were determined by tests of nonsyphilitic serums at 1 to 2 C. and low titer syphilitic serums at room temperature. It was found that a mixture containing 2 mg. cardiolipin and from 18 to 20 mg. lecithin per cc. was optimal for use in the slide test;⁵ 0.1 cc. of this mixture was used exactly as was 0.1 cc. of Kline antigen in the preparation of the diagnostic emulsion. The emulsion with cardiolipin-lecithin antigen required no heating before use.

The superiority in specificity of cardiolipin-lecithin antigen was strikingly shown in tests of the blood of nonsyphilitic individuals with malaria, reported previously⁵ (See Table 1).

The following report concerns a clinical and serologic evaluation of 27,103 consecutive slide tests with optimal cardiolipin-lecithin antigen and Kline antigen in general hospital and ambulatory patients and in individuals presenting themselves for prenatal and premarital examinations. The tests were performed between June 26, 1945 and October 1, 1947 (a period of twenty-seven months), and in the course of the study, five different lots of cardiolipin solution and lecithin solution were used.

Table 2 shows the comparative specificity of optimal cardiolipin-lecithin antigen and Kline diagnostic and exclusion antigen emulsions in the slide test.

As will be seen from Table 2, the specificity of optimal cardiolipin-leeithin emulsion is much greater than that of Kline diagnostic and exclusion antigen emulsions. The remarkably small number of false positive reactions with

* Presented at the Twenty-Sixth Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 29, 1947. Received for publication, October 29, 1947.

cardiolipin-lecithin antigen (7 in 24,877 tests of nonsyphilitic blood, the majority from general hospital patients) indicates clearly that no greater specificity in a test for syphilis is likely to be achieved.

TABLE 1*

NEGATIVE AND FALSE-POSITIVE REACTIONS WITH VARIOUS ANTIGENS IN CASES OF MALARIA (CENTRIFUGED EMULSIONS)

READING	ANTIGEN					
	Eagle	Hinton	Kahn	Kline	Mazzini	Cardiolipin†
Negative.....	87	90	93	91	77	100
False reactions.....						
±.....	3	2	2	2	10	0
+.....	11	9	6	8	14	1
Total.....	14	11	8	10	24	1
Total tests.....	101	101	101	101	101	101
Negative.....	108	111		112	97	126
False reactions.....						
±.....	5	4		3	13	0
+.....	14	12		12	17	1
Total.....	19	16		15	30	1
Total tests.....	127	127		127	127	127
Negative.....		132		133	118	150
False reactions.....						
±.....		6		5	15	1
+.....		14		14	19	1
Total.....		20		19	34	2
Total tests.....		152		152	152	152
Negative.....				151	132	176
False reactions.....						
±.....				7	17	1
+.....				20	29	1
Total.....				27	46	2
Total tests.....				178	178	178
Negative.....				223		265
False reactions.....						
±.....				12		2
+.....				35		3
Total.....				47		5
Total tests.....				270		270

* Reprinted from Am. J. Clin. Path., 16: 73, 1946.

† The majority of tests were performed with a mixture of 1 part cardiolipin to 10.6 parts lecithin (optimal). All cardiolipin mixtures used gave more sensitive results in cases of syphilis than did Kline antigen.

Table 3 gives clinical data of the cases with false-positive reactions.

As will be seen from Table 3, the incidence of false-positive reactions with cardiolipin-lecithin antigen in various diseases and conditions is remarkably

TABLE 2
FALSE POSITIVE REACTIONS WITH OPTIMAL CARDIOLIPIN-LECITHIN AND
KLINE ANTIGEN EMULSIONS IN 24,511 NONSYPHILITIC SERUMS*

	ANTIGEN EMULSIONS		
	Optimal Cardiolipin- Lecithin	Kline Diagnostic	Kline Exclusion
False positive tests (++ to ++++)	7 0.028%	67 0.27%	231 0.93%
False weakly positive tests (± to +)	49 0.2%	147 0.59%	281 1.13%

* Total tests June 26, 1945 to October 1, 1947, 27,103; total syphilitic serums, 2466 (9.1%); tests in cases of questionable syphilis, 26; tests in cases with insufficient data, 100.

TABLE 3
CLINICAL DATA OF CASES WITH FALSE POSITIVE SLIDE TEST REACTIONS

DISEASE OR CONDITION	TOTAL TESTS	FALSE POSITIVE TESTS (++ to ++++)			FALSE WEAKLY POSITIVE TESTS (± to +)		
		OCL	KD	KE	OCL	KD	KE
Infectious mononucleosis.....	56	1	2	6	0	2	1
Viral respiratory infection.....	247	1	8	15	1	5	2
Seborrheic eczema*.....		2	0	0	2	0	0
Jaundice.....		1	3	4	0	1	2
Carcinoma.....	513	1 (0.2%)	0	6 (1.2%)	3 (0.6%)	4 (0.8%)	1 (0.2%)
Insignificant or no disease.....		1	39	75	19	19	8
Pregnancy.....	4313	0	8 (0.2%)	25 (0.6%)	6 (0.14%)	11 (0.26%)	11 (0.26%)
Fracture.....	659	0	0	5 (0.8%)	4 (0.6%)	4 (0.6%)	0
Vaccination.....		0	1	3	2	2	2
Diabetes.....	313	0	0	4	4	1	3
Coronary occlusion.....	129	0	0	5	3	2	2
Abortion.....	420	0	3	6	2	3	0
Malaria.....		0	0	2	1	2	0
Rectal abscess.....	41	0	3	3	0	0	0
Lymphadenitis.....		0	0	2	0	2	1
Peptic ulcer.....		0	0	0	2	0	0
Tuberculosis of bone.....		0	0	1	0	2	1
Bone infection.....		0	0	1	0	1	1
Viral infection of bowel.....		0	0	1	0	1	0
Herpes.....	14	0	0	1	0	0	0
Totals.....		7	67	165	49	62	35

OCL, Optimal cardiolipin-lecithin; KD, Kline diagnostic; KE, Kline exclusion.

* All four tests from same patient.

TABLE 4
COMPARATIVE SENSITIVITY OF OPTIMAL CARDIOLIPIN-LECITHIN ANTIGEN
AND KLINE ANTIGEN IN SLIDE TESTS OF SYPHILITIC SERUMS

ANTIGEN	READINGS							TOTAL TESTS
Optimal cardiolipin- lecithin.....	++ to ++++	± to +	++ to ++++	—	++ to ++++	± to +	—	
Kline diagnostic.....	++ to ++++	± to +	—	++ to ++++	± to +	—	± to +	
Number of tests...	1240	26	270	1*	460	249	0	2246
Percentage.....	55.2	1.2	12	.04	20.4	11.1	0	100.
Optimal cardiolipin- lecithin.....	++ to ++++	± to +	++ to ++++	—	++ to ++++	± to +	—	
Kline exclusion.....	++ to ++++	± to +	—	++ to ++++	± to +	—	± to +	
Number of tests....	1727	146	41	0	217	103	1*	2235
Percentage.....	77.3	6.5	1.8	0	9.7	4.6	.04	100.

* These two cases were judged by the clinicians to have had more than adequate treatment.

TABLE 5
COMPARATIVE SENSITIVITY OF OPTIMAL CARDIOLIPIN-LECITHIN ANTIGEN AND KLINE
ANTIGEN IN SLIDE TESTS OF THE NEWBORN OF 5 TREATED SYPHILITIC MOTHERS

INITIALS OF MOTHER	DATE	SPECIMEN OF BLOOD	RESULT OF TESTS WITH		
			Optimal Cardiolipin- Lecithin	Kline Diagnostic	Kline Exclusion
D. G.	12-13-45	Uterine	++++	++++	++++
	12-13-45	Cord	++++	—	—
	1-19-46	Infant	+++	—	—
	3-14-46	Infant	Pos.		
	4- 9-46	Infant	Pos.		
	5-17-46	Infant	—		
	6-20-46	Infant	—		
A. W.	3- 6-46	Uterine	++++	++	++++
	3- 6-46	Cord	++++	—	—
	3- 7-46	Infant	++++	—	—
E. P.	8- 3-46	Uterine	++++	+	++++
	8- 3-46	Cord	++++	++++	++++
	10- 2-46	Mother	++++	++++	++++
	10- 2-46	Infant	+++	—	—
	10-30-46	Infant	—	—	—
	8- 6-47	Infant	—	—	—
T. Z.	12- 5-46	Prenatal	++++	++	++++
	5- 6-47	Uterine	++++	++++	++++
	5- 6-47	Cord	++++	—	—
V. J.	1- 9-47	Prenatal	++++	++++	++++
	6-16-47	Mother	++++	+	++++
	6-19-47	Infant	++	—	—
	10-21-47	Infant	—	—	—

low. Only 1 false-positive reaction occurred in 487 tests of blood from patients with cancer, none in 4158 tests of pregnant patients and none in 659 tests of patients with fracture. Only 1 of 51 tests of patients with infectious mononucleosis gave a false-positive reaction; 2 positive and 2 weakly positive reactions were obtained in a case of seborrheic eczema. Some of the nonspecific weakly positive reactions with cardiolipin-lecithin antigen occurred in tests of uterine and cord bloods. Subsequent tests of venous bloods were negative.

TABLE 6

TITRATION OF THE SERUM OF A PENICILLIN-TREATED CASE (R. K.) OF SECONDARY SYPHILIS

	SERUM DILUTION	1944			1945		1946		1947	
		9/7	10/31	12/20	3/28	7/11	2/14	11/1	5/22	7/31
Optimal cardio- lipin-lecithin antigen emul- sion	0					++++	+	±	—	—
	1:2					±	—	—		
	1:4					—				
Kline exclusion antigen emul- sion	0	++++	++++	++++	++++	+	±	—	—	—
	1:2	++++	++++	++++	++	—	—	—		
	1:4	++++	++++	++++	—	—				
	1:8	++++	++++	++++						
	1:16	++++	++++	—						
	1:32	++++	++++							
	1:64	++++	++++							
	1:128	++++	±							
	1:256	++++	—							
	1:512	++++	—							
	1:1024	++++	—							
	1:2048	—	—							
Kline diagnos- tic antigen emulsion	0	++++	++++	++++	±	—	—	—	—	—
	1:2	++++	++++	++++	—	—	—	—		
	1:4	++++	++++	+++	—	—				
	1:8	++++	++++	—						
	1:16	++++	++++	—						
	1:32	++++	—							
	1:64	++++	—							
	1:128	++++	—							
	1:256	—	—							

Table 4 shows the comparative sensitivity of optimal cardiolipin-lecithin antigen and Kline antigen in slide tests of syphilitic serums.

Table 4 shows that optimal cardiolipin-lecithin antigen emulsion gives results in syphilitic serums which are not only much more sensitive than those obtained with Kline diagnostic antigen emulsion but also are definitely more sensitive than those obtained with Kline exclusion antigen emulsion.

Table 5 shows the striking specificity and sensitivity of optimal cardiolipin-lecithin antigen emulsion for syphilitic reagin in the blood of the newborn of treated syphilitic mothers.

Table 6 shows that the optimal eardiolipin-leeithin antigen emulsion possesses greater sensitivity in a treated case of syphilis than do the Kline antigen emulsions.

SUMMARY

Clinical evaluation of 27,103 comparative slide tests for syphilis with eardiolipin-leeithin antigen and Kline antigen shows:

1. Optimal eardiolipin-leeithin antigen emulsion gives results, in the slide test for syphilis, of decidedly greater specificity and much greater sensitivity than does diagnostic Kline antigen emulsion and, therefore, is of greater value than the latter in the diagnosis of the disease.

2. Optimal eardiolipin-leeithin antigen emulsion gives results, in the slide test, of decidedly greater sensitivity in syphilitic serums and much greater specificity in nonsyphilitic serums than does Kline exclusion antigen emulsion and therefore, when negative, serves better than the latter in the exclusion of syphilis.

3. Optimal eardiolipin-leeithin antigen, having been found to give results of maximum specificity and maximum sensitivity, is now being used as the standard antigen for the Kline test. The Kline antigen is employed as an adjunct of confirmatory value.

Acknowledgment. We wish to thank Miss Mary Stephens, M.T. (ASCP), for her excellent technical assistance in the performance of many of the tests.

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THE V.D.R.L. SLIDE TEST

A COMPARISON WITH THE MAZZINI, KAHN AND KOLMER TESTS FOR SYPHILIS*

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Cardiolipin was isolated from beef heart by Pangborn, in 1941,⁹ and was classified by her as a complex phosphatidic acid.¹² Various mixtures of cardiolipin with a purified lecithin¹¹ and cholesterol have been suggested by a number of investigators^{1-3, 5, 6, 8, 13} as substitutes for the crude lipoidal antigens now employed in the serologic tests for syphilis.

The cardiolipin-lecithin-cholesterol antigens have been considered to be at least as specific as the crude antigens now used in the standard tests for syphilis.^{1, 2, 6, 8, 13, 14}

In 1946, Harris, Rosenberg and Riedel³ reported their microflocculation test for syphilis, the V.D.R.L. (Venereal Disease Research Laboratory) slide test, using a cardiolipin-lecithin-cholesterol mixture. A comparison will be made in this article of the reactivity of this antigen as used in the V.D.R.L. slide test with three other tests routinely used in the Bureau of Laboratories, namely, the Mazzini and Kahn flocculation tests^{4, 9} and the Kolmer complement-fixation test.⁷

METHOD

The blood specimens tested were unselected, routine serums submitted to the New York City Bureau of Laboratories by the physicians of the city for a variety of reasons, such as diagnosis, tests before employment or marriage and for pre-natal care.

All specimens were first tested by the Mazzini slide test. All serums yielding positive or doubtful reactions with the Mazzini test were further studied by the Kahn and Kolmer techniques, as well as by the V.D.R.L. slide test.†

To determine the relative efficiency of test performances in the Bureau of Laboratories, duplicate specimens of blood were tested each week by this laboratory and by the United States Public Health Service Venereal Disease Research Laboratory, at Stapleton, Staten Island, New York.

The results obtained on approximately 300 such specimens (Table 1) indicate that the reactivity levels of the four procedures were similar in the two laboratories.

RESULTS

It was found that of 52,372 routine specimens examined by the Bureau of Laboratories, 8519 (16.2 per cent) were Mazzini reactors (Table 2).

* Received for publication, December 2, 1947.

† The V.D.R.L. antigen used in this study was kindly furnished by the Venereal Disease Research Laboratory, U. S. Public Health Service, Stapleton, Staten Island, New York.

Of the Mazzini reacting serums, 83.6 per cent were confirmed by the V.D.R.L. slide test, 64.2 per cent by the Kolmer and 57.4 per cent by the Kahn technics.

Since the Mazzini slide test is employed as a screen test by the Bureau of Laboratories, it was necessary to determine the number of serums that reacted by the V.D.R.L. slide technic, but were reported "negative" by the Mazzini test.

Of 8425 serums that were "negative" by the Mazzini technic, 74 (0.8 per cent) were V.D.R.L. slide test reactors. The Kolmer complement-fixation test produced approximately the same number of reactors in this group.

TABLE 1
SPLIT SPECIMENS TESTED BY NEW YORK CITY AND THE
VENEREAL DISEASE RESEARCH LABORATORY

TEST	NUMBER OF SPECIMENS	POSITIVE		DOUBTFUL		NEGATIVE	
		N.Y.C.	U.S.P.H.S.	N.Y.C.	U.S.P.H.S.	N.Y.C.	U.S.P.H.S.
Mazzini.....	303	67	64	25	30	211	209
Kahn.....	280	44	40	6	7	230	233
Kolmer.....	286	59	60	5	2	220	221
V.D.R.L.....	320	93	96	24*	21*	203	203

* Reported as "weakly positive".

TABLE 2
COMPARISON OF REACTIVITY OF MAZZINI, KAHN AND KOLMER TESTS
AND V.D.R.L. SLIDE TESTS IN 52,372 ROUTINE SPECIMENS

TEST	NUMBER OF REACTORS	PER CENT OF TOTAL	PER CENT OF MAZZINI REACTORS
Mazzini.....	8519	16.2	—
Kahn.....	4891	9.3	57.4
Kolmer.....	5449	10.4	64.2
V.D.R.L.....	7145	13.6	83.6

It is therefore apparent that the Mazzini slide test is suitable as a screen test for a large public health laboratory where a considerable number of specimens are tested daily, and that very few V.D.R.L. reactors (as well as Kolmer reactors) are missed by this procedure.

Agreement and Disagreement among the Four Tests for Syphilis

The reactivity of individual specimens by the Mazzini, V.D.R.L. and Kahn flocculation and by the Kolmer complement-fixation tests was analyzed. Approximately 50 per cent of the Mazzini reactors tested by this laboratory also reacted by the other three technics. The remainder of the specimens were "negative" by one or more of the tests employed (Table 3).

It previously has been demonstrated¹⁵ that about 25 to 30 per cent of the Mazzini reactors are negative by the Kahn and Kolmer technics. The data in Table 3 confirm these findings and in addition show that less than one-half of

these specimens are also negative by the V.D.R.L. slide test (see groups IV and V, Table 3). The V.D.R.L. slide test, therefore, confirms more than one-half of the Mazzini reactors that were reported as negative by both the Kahn and Kolmer tests (Group IV, Table 3).

Upon examination of the serums that reacted by the two slide tests, but were "negative" by the Kahn and Kolmer techniques, it was found that a greater number of "positive" results were recorded by the V.D.R.L. slide test than by the Mazzini slide test. These results are summarized in Table 4.

TABLE 3

COMPARISON OF 8519 V.D.R.L. SLIDE TESTS WITH MAZZINI, KAHN AND KOLMER TESTS.
SPECIMENS WERE MAZZINI REACTORS "SCREENED" FROM 52,372 ROUTINE SERUMS

GROUP	SEROLOGIC RESULT*	NUMBER OF SPECIMENS	PER CENT OF MAZZINI REACTORS
I	M+ V+ K+ KOL+	4326	50.7
II	M+ V+ K+ KOL-	457	5.3
III	M+ V+ K- KOL+	912	10.7
IV	M+ V+ K- KOL-	1450	17.2
V	M+ V- K- KOL-	1110	13.0
VI	M+ V- K- KOL+	156	1.8
VII	M+ V- K+ KOL-	53	0.6
VIII	M+ V- K+ KOL+	55	0.6
Total		8519	

* M, Mazzini; V, V.D.R.L.; K, Kahn; KOL, Kolmer; +, Reactor; -, Negative.

TABLE 4

"REACTORS" BY MAZZINI AND V.D.R.L. SLIDE TESTS
NEGATIVE BY KAHN AND KOLMER TESTS
488 SPECIMENS

TEST	POSITIVE	PER CENT	DOUBTFUL	PER CENT
Mazzini	138	28.2	350	71.8
V.D.R.L.	184	37.7	304*	62.3

* The V.D.R.L. slide test reports these as "weakly positive".

Since the Mazzini slide test is employed by the New York City Bureau of Laboratories as a screen test, the V.D.R.L. slide test was compared with the two diagnostic tests, the Kahn and Kolmer. Table 5 reveals the extent of agreement and disagreement in these three tests.

In order to compare further the V.D.R.L. slide test with the Mazzini, Kahn and Kolmer techniques, the laboratory reports of a venereal disease clinic were analyzed.

Blood specimens from patients of the Bureau of Social Hygiene, New York City Department of Health, were divided into two categories: medical advisory (MA) and known syphilitics (RX) for the purpose of this study.

The data for this group of serums are given in Table 6. It will be seen that

approximately 17 per cent of the specimens of known syphilitics under treatment and 18.3 per cent of the medical advisory group were reported "negative" by the Kahn and Kolmer tests, but reacted in the Mazzini and V.D.R.L. tests. A further analysis of the data presented in Table 6 would indicate that the Mazzini slide test retains its reactivity in known syphilitics under treatment

TABLE 5

AGREEMENT AND DISAGREEMENT OF RESULTS ON 7409 MAZZINI REACTORS TESTED BY V.D.R.L. SLIDE, KAHN STANDARD AND KOLMER COMPLEMENT-FIXATION TESTS

SEROLOGIC REACTION*	NUMBER OF SPECIMENS	PER CENT OF TOTAL
V+ K+ KOL+	4326	58.3
V+ K+ KOL-	457	6.2
V+ K- KOL+	912	12.3
V+ K- KOL-	1450	19.5
V- K- KOL+	156	2.1
V- K+ KOL-	53	0.7
V- K+ KOL+	55	0.7
Total	7409	

* V, V.D.R.L. slide test; K, Kahn standard test; KOL, Kolmer complement-fixation test; +, reactor; -, negative.

TABLE 6

SEROLOGIC REACTIONS OF 1210 SPECIMENS FROM KNOWN SYPHILITICS (RX) AND MEDICAL ADVISORY PATIENTS (MA) OF A VENEREAL DISEASE CLINIC

GROUP	REACTION*	MA	MA REACTORS	RX	RX REACTORS
			<i>per cent</i>		<i>per cent</i>
I	M+ V+ K+ KOL+	420	53.5	235	55.3
II	M+ V+ K+ KOL-	64	8.1	37	8.7
III	M+ V+ K- KOL+	83	10.5	65	15.2
IV	M+ V+ K- KOL-	144	18.3	71	16.7
V	M+ V- K- KOL-	74	9.4	17	4.0
Total		785	42.5		

* M, Mazzini; K, Kahn; KOL, Kolmer; +, reactor; -, negative.

for a longer period than does the V.D.R.L. slide test (compare the MA and RX of Group V, Table 6).

DISCUSSION

The V.D.R.L. slide test employs as an antigen the eardiolipin-lecithin-cholesterol formula as described by Harris, Rosenberg and Riedel.³ The reactivity of this test was compared with the Mazzini and Kahn flocculation and the Kolmer complement-fixation tests in more than 50,000 routine specimens "screened" by the Mazzini slide test. The Mazzini reactors, *i.e.*, all positive or

doubtful reacting specimens, were further tested by the V.D.R.L. slide, the Kahn Standard and the Kolmer complement-fixation tests.

The reliability of the Mazzini slide test as a screen test in a large public health laboratory is evidenced by the fact that of 8425 "negative" Mazzini serums only 74 (0.8 per cent) were V.D.R.L. slide test reactors. The Kolmer complement-fixation test produced approximately the same number of reactors in this group.

The relative reactivity of the V.D.R.L. slide, the Kahn Standard and the Kolmer complement-fixation tests was 83.6 per cent, 57.4 per cent and 64.2 per cent, respectively, of the Mazzini reactors tested.

In a previous report¹⁵ it was shown that about 25 per cent of the Mazzini reactors are unconfirmed by the Kahn and Kolmer tests. More than one-half of this group reacted in the V.D.R.L. slide test (Table 4).

In a comparison of the V.D.R.L. and the Mazzini slide tests, it is seen that the former reports a larger number of "positives" (Table 4) and a larger number of "negatives". In other words, the "doubtful" Mazzini reactors are often either "positive" or "negative" V.D.R.L. reactors.

A comparison of the V.D.R.L. slide test with the Standard Kahn and the Kolmer complement-fixation tests indicates (Table 5) that approximately 60 per cent, of the specimens studied showed positive or doubtful reactions in all three tests. The remaining specimens were "negative" by one or two of the tests employed. The percentage of "negative" reports for the Kahn, Kolmer and V.D.R.L. tests were 33.9 per cent, 26.4 per cent and 3.5 per cent, respectively, (Table 5). Only those specimens that reacted in one of the three diagnostic tests were included in this group.

No attempt has been made, in this report, to compare serologic and clinical findings. At present, we are simply comparing, the reactivity of a new test, the V.D.R.L. slide test, which employs a cardiolipin-lecithin-cholesterol mixture, with the three standard tests which use lipoidal antigens.

SUMMARY

The V.D.R.L. slide test, which employs a cardiolipin-lecithin-cholesterol antigen, was compared for reactivity with the Mazzini and Kahn flocculation, and the Kolmer complement-fixation tests in more than 50,000 routine specimens submitted to the Bureau of Laboratories.

The sensitivity of the four tests, in order of reactivity was: the Mazzini slide test, the V.D.R.L. slide test, the Kolmer complement-fixation test and the Standard Kahn test.

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CLINICOPATHOLOGIC CONFERENCE*

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CLINICAL DATA

History. A 21 year old housewife entered the hospital complaining of pain in the abdomen. About two months before admission the patient suffered a sudden attack of sharp midabdominal pain which radiated into the chest and to the apices of both shoulders. There had been no premonitory symptoms. During this attack she noted a tender mass to the left of her umbilicus. The pain was intermittent in character and continued for two days with diminishing severity. At the end of this period both pain and mass disappeared. Thereafter she was able to elicit tenderness on deep pressure but save for some fatigue remained asymptomatic.

Three days prior to entry, there was an abrupt onset of an identical episode and again a "lump" was felt in the left side of the abdomen. The pain was agonizing although intermittent. There was neither nausea nor constipation, but on the night before admission she vomited precipitously several times. The vomitus was not blood- or bile-stained. Standing erect caused the pain to grow worse. At first, lying down produced complete relief, but later this only caused the knifelike intensity to become dulled, although nonetheless severe. During the day preceding admission, the mass was noted to increase in size. Bowel movements were unchanged in frequency or appearance.

The patient had one child, two years of age. The past and family histories were noncontributory. Menses were regular, the last period having terminated three weeks before the onset of the present attack.

Physical examination showed a thin, tired-looking young woman, lying quietly in bed, complaining apprehensively of deep-seated left-sided abdominal pain. The facies revealed anxiety. There was no icterus. The cardiac findings were normal; the pulse rate was 60 and the blood pressure 115/65. The lungs were clear and the respiratory rate was 22.

The abdomen appeared wasted and flabby. At occasional intervals, irregular peristaltic movements were visible. Just to the left of the umbilicus a lump was visible and this proved to be a tender, firm, rounded, freely movable mass measuring approximately 8 x 5 cm. The upper portion of the abdomen was tender on deep palpation, but there was no rigidity. The liver and spleen were not enlarged and the extremities and neurologic examination were negative. The temperature was 98.6 F.

Examination of the blood showed an erythrocyte count of five million with 10.6 Gm. hemoglobin per 100 ml. The leukocytes numbered 26,400 with 90 per cent neutrophils, of which 41 per cent were band forms. The red blood

* Received for publication, December 5, 1947.

cell sedimentation rate (Wintrobe) was 28 mm. per hour. The urine showed an occasional white blood cell, but was otherwise negative. A Kahn test was negative.

Plain roentgen films of the abdomen, both in the erect and prone positions, were negative. Pyelograms after intravenous injection of the dye, were also negative.

During the initial days in the hospital there was little change in the abdominal findings. The mass remained palpable, exquisitely tender and appeared to become slightly larger. There were several exacerbations of abdominal pain of such intensity as to cause the patient to thrash about in bed. In the main, however, the distress was dull and persistent in character. On the second hospital day the patient had a chill which was followed by a rise in temperature to 102.6 F. The fever subsided after twenty-four hours and thereafter only an occasional rise to 99.5 F. occurred. Urinalysis on the third day showed a trace of albumin; the sediment contained 8 to 10 red blood cells and 10 to 12 white blood cells per high power field. The leukocyte count remained at 26,000 per cu. mm. Therapy consisted of bed rest, soft and liquid diet, opiates, sulfadiazine and penicillin. Several soft, partly formed, brown stools were passed spontaneously. There was no dysuria. On the eighth hospital day, the leukocyte count was 8000 with 63 per cent neutrophils, 29 per cent lymphocytes, 4 per cent eosinophils and 4 per cent monocytes.

Two days later an operation was performed.

CLINICAL DISCUSSION

Dr. Leon Schiff. "Dr. Felson, would you review the films?"

Dr. Benjamin Felson. "I'm afraid I haven't very much help to offer you. The plain and upright films of the abdomen show no soft tissue mass. The colon is fairly well outlined with gas and, as you can see here, there is no displacement. The psoas and kidney outlines are not well shown on these films. There are no unusual shadows in the hepatic or splenic areas and no calcifications are seen. In the upright films, the lower portions of the lung fields and the heart appear normal. In the 'intravenous' pyelograms there is normal excretion of dye and excellent visualization of both pelves and ureters. No abnormalities appear on either side."

Dr. Leon Schiff. "The presence of midabdominal pain accompanied by a mass to the left of the umbilicus suggests the possibility of a pancreatic cyst secondary to pancreatitis. The fact that the abdominal pain and the appearance of the mass occurred so closely together, however, leads me to exclude a pancreatic cyst of either neoplastic or pseudocystic character as the cause of the patient's symptoms. A serum amylase might have been helpful, but such a determination, unfortunately, was not made. The sudden onset of the pain and its radiation to the apex of both shoulders suggests diaphragmatic irritation either through the leakage of blood or by reason of the occurrence of secondary infection.

"The fact that the mass was freely movable and located to the left of the

umbilicus is quite in keeping with a diagnosis of mesenteric cyst. Since mesenteric cysts are rarely pedunculated, pain associated with such a cyst would have to be explained on the basis of hemorrhage into the cyst rather than by torsion of its pedicle. The curious radiation of the pain would have to be explained by leakage of blood from the cyst, although the absence of a more impressive anemia militates somewhat against such an assumption. The recurrence of the pain two months after the initial attack and the increase in the size of the mass could be explained by a second hemorrhage into its substance. The disappearance of the mass in the interval between attacks could result from the leakage of its contents into the lesser peritoneal sac, in which case there would be no signs of generalized peritonitis. An ovarian cyst with a twisted pedicle seems unlikely in view of the location of the mass, but more particularly because of the radiation of the pain into the chest. I must admit that I am very unhappy about any diagnosis I am able to make.

"My impression is that the mass was a mesenteric cyst with recurrent intracystic hemorrhage and leakage into the lesser peritoneal sac."

Dr. B. Felson. "What evidence have you that this was not a pancreatic cyst with partial evacuation into the lesser sac?"

Dr. Schiff. "This mass became noticeable simultaneously with the pain and from the description given may even have been present before symptoms directed attention to it. Cysts of the pancreas are usually pseudocysts secondary to some intrapancreatic inflammatory disorder and there is almost invariably an interval of some days intervening between the abdominal pain and the appearance of the mass. I am inclined to believe this mass antedated the initial episode of pain, but I feel unhappy about the complete disappearance of the tumor between the two attacks. Dr. Zininger, what is your thought about this?"

Dr. M. M. Zininger. "This case presents a puzzling diagnostic problem to me, as I imagine it undoubtedly does to others here. The radiation of the pain in the first attack to the back and tips of both shoulders, suggests that the lesion was located in the upper part of the abdomen and possibly in the retroperitoneal region. This may not be significant, however, in view of the fact that subsequently the pain seems to have been localized in the tumor or in the region of the tumor.

"My first impression on hearing the story of a disappearing painful tumor was that it might have been a hernia, or a recurrent intussusception but the absence of signs of intestinal obstruction seems to preclude those lesions. The fact that the patient was able to eat a soft diet and have normal bowel movements during her ten-day stay in the hospital practically rules out, to my mind, a lesion of stomach or intestine. The severe, cutting pain may be of help in diagnosis. Severe pain of this type may occur suddenly in several situations but chiefly in one of the following three: (1) sudden hemorrhage into a solid tumor or cyst; (2) sudden distention of a hollow viscus; and (3) ischemia such as occurs with volvulus or twist of the pedicle of a cyst or tumor. It is my belief that the third of these is accompanied by the most severe pain, such as was present in this patient, and it is therefore my guess that that was the type of

lesion present here. Such a lesion might, as in this case, show a disappearing tumor. If the twist remained, we would expect generalized abdominal pain from extravasated blood. There would also be leukocytosis without much fever, due to absorption of degenerative products as the tumor or cyst underwent ischemic necrosis. Also, as necrosis occurs the pain often becomes less intense.

"Considering the location of the tumor, namely, to the left of the umbilicus, not many possibilities present themselves. A pancreatic tumor or cyst should not be so freely movable, nor should it disappear. A mesenteric cyst should not be painful, nor should it disappear. The torsion of an abnormally mobile spleen or kidney could give such a syndrome, but the spleen is said to be normal, and the kidneys normal by intravenous pyelogram. Torsion of the omentum might be considered, but seems unlikely as it rarely forms a definite mass, never a disappearing tumor, and usually occurs in fat persons following exercise. It seems to me that the most likely point of origin of the tumor is the pelvis, for it is well known that pelvic lesions may be present in the abdomen. We are not told in the protocol about the findings on pelvic examination, but they may not have helped in the diagnosis, because frequently tumors of pelvic origin lying in the abdomen may seem to be entirely free of pelvic connection on bimanual examination."

Dr. Edward A. Gall. "A pelvic examination was not recorded."

Dr. Zininger. "That's usually the case, isn't it? At all events, the best suggestion I can make as to diagnosis is either an ovarian cyst, or a pedunculated fibroid of the uterus with a twisted pedicle. Several years ago I operated upon a young woman who had had recurrent attacks of severe left lower abdominal pain and a disappearing tumor somewhat similar to the mass in this case. Operation disclosed a pedunculated fibroid of the uterus which showed evidence of having been twisted on its pedicle. I attributed the pain in the shoulders to bloody extravasation. The transitory hematuria may be due to a degenerated mass lying on the ureter."

Dr. Gall. "Are there any other comments?"

Dr. David Graller. "I still think this could be a pseudocyst of the pancreas. But if such a cyst had leaked, there should have been much more evidence of peritoneal irritation."

Dr. Schiff. "Even if the leak had occurred into the lesser peritoneal sac?"

Dr. Graller. "No, I suppose under such circumstances the evidence of peritonitis would be minimal."

Dr. B. Felson. "I don't think any of the pancreatic cysts we've seen have been mobile, certainly not as freely movable as this one. On the other hand we have seen one mesenteric cyst which visualized roentgenographically in the left paraspinal region, but which disappeared a few days later."

Dr. Zininger. "I've seen many mesenteric cysts but none with pain. They disappear sometimes because the patient loses track of them, but I've never seen one empty spontaneously. They are usually freely movable."

Dr. Carl W. Kumpe. "How do you account for the shoulder pain?"

Dr. Schiff. "I thought that the seepage of blood under the diaphragm could

explain that. If there had been frank perforation, I should think fever would have occurred earlier and the abdominal signs would have been much more generalized."

Dr. Zinninger. "The history cites two identical episodes so that presumably there was shoulder pain during both attacks."

Dr. Gall. "That is correct."

Dr. Zinninger. "That would certainly lead one to suspect a lesion in the upper abdomen."

Dr. Schiff. "The mass or something associated with it, such as seepage of blood could lie in relation to the diaphragm. You do have this sort of referred pain in association with perforated ulcer, of course."

Dr. Zinninger. "There is no progression of symptoms such as one would anticipate with the expulsion of irritative material into the free peritoneal cavity. Both episodes were drastic and there was a high degree of leukocytosis with relatively little fever. That suggests to me necrosis of tissue due to ischemia. You get leukocytosis without fever of parallel degree in infarction of a viscus."

Dr. Schiff. "But you may also have that with hemorrhage."

Dr. Gall. "What is your opinion, Dr. Felson?"

Dr. Henry Felson. "I agree with Dr. Zinninger. This strikes me as being more on the order of an infarction of a cyst or pedunculated tumor than it does perforation. It would be difficult to fit the two episodes into the diagnosis of cyst unless one presumed that the cyst refilled and ruptured a second time, or that the initial symptoms were due to an incomplete rupture followed after two months by a major perforation. I think with the information available that that is unlikely."

Dr. MacDonald Wood. "I should like to offer two other possibilities: a congenital reduplication of the colon with volvulus or a diverticulum of the stomach."

Dr. Kumpe. "Don't you believe that the absence of any significant gastrointestinal symptoms would tend to rule out such diagnoses? I think the history and findings here are very similar to those which we discussed in another patient who proved to have a pancreatic pseudocyst due to pancreatitis."

Dr. Gall. "Are there other suggestions?"

PATHOLOGIC DISCUSSION

Dr. Gall. "I saw this patient clinically and in the face of a rounded, tender, relatively freely movable mass in the midabdomen, made a diagnosis of twisted ovarian cyst. The surgeon's pre-operative diagnosis was abdominal tumor, type and origin unknown.

"The patient was operated on through a left rectus incision directly over the mass. As soon as the peritoneal cavity was entered, the mass was encountered. It was found to be bound by thin, fibrous and fibrinous adhesions to the omentum which completely encased it. The omentum was partially dissected from it and the tumor was found to be the spleen, freely movable and ectopic. It was connected to the upper abdomen by a long pedicle composed in the main of the

splenic vessels. The pedicle was twisted and both the arterial and venous channels were thrombosed.

"Part of the omentum and the spleen with a short stump of its pedicle were excised and the abdomen closed without drainage. Postoperatively the patient continued to have mild pain in both shoulders for several days. This gradually subsided, however, and she was discharged symptom-free on the tenth post-operative day.

"Microscopic studies showed complete occlusion of hilar vessels by thrombi which exhibited evidence of early organization. The splenic substance was massively infarcted except for a thin rind beneath the capsule, the viability of which was undoubtedly preserved through the medium of the small vessels evident in the attached adhesive strands which extended to the capsule from the adherent omentum."

ANATOMIC DIAGNOSES

1. Aberrant spleen with torsion of pedicle,
2. Thrombosis of splenic artery and veins,
3. Massive infarction of spleen.

EDITORIALS

CARDIOLIPIN

Six years ago cardiolipin, as a new phospholipin component of antigen for serologic tests for syphilis, was announced. Clinicians and serologists welcomed this announcement for they hoped that this new substance would be the long-sought-for key to the simplification or, at least, to the clarification of the serology of syphilis. They thought that simplification would be achieved by the adoption of a few universally accepted procedures which would utilize cardiolipin. Thus, the innumerable procedures which used the lipoidal antigens would be eliminated.

But six years of experience shows that cardiolipin has neither simplified nor clarified the serology of syphilis. Cardiolipin antigens have been adapted to the technics which formerly used lipoidal antigens. The number of technical methods has increased rather than decreased. These new methods are being used by ever increasing numbers of laboratories.

In the testing of large numbers of specimens from syphilitic individuals, the performance of cardiolipin antigens has been comparable to that of the older antigens; but as yet there has not been sufficient testing of material from diverse nonsyphilitic individuals to warrant any conclusion concerning the specificity of the modified procedures. Final appraisal of cardiolipin can be achieved only when it has been applied to a significant volume of authenticated, clinically diverse testing material. Perhaps this appraisal will be achieved from the analysis of the mass of data accumulating from the extensive testing now in progress. If not, then the measure of specific reliability must be determined by the more cumbersome procedure of original method evaluation studies.

To put it simply, although laboratory observations justify much optimism concerning the future of cardiolipin in serologic tests for syphilis, it has not been demonstrated that its use can remove any responsibility from the clinician who interprets the results of laboratory procedures in behalf of the patient for whom the tests are performed.

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LABORATORY TRAINING FOR RESIDENTS IN THE SPECIALTIES

Certification Boards in the specialties, as a rule, require that six months of the three year period of graduate institutional training be spent in a hospital laboratory where the candidates are to receive instruction in the basic medical sciences. The requirements provide that the instruction be divided into anatomy, 40 per cent; pathology, 40 per cent; physiology and chemistry, 10 per cent; and bacteriology, 10 per cent. The instructions further suggest that the time assigned to the laboratory may be full time for six months, part time for one year, or a few hours each week throughout the entire training period. In theory

these provisions seem to be satisfactory, but in practice they give too much leeway for individual variation and/or, in some instances, for evasion. This point was emphasized in the discussion of a paper on "Graduate Medical Training of Residents" presented at the September meeting of the Pennsylvania State Clinical Pathological Society, where it was brought out that clinical department heads usually choose the third plan because it does not completely interrupt the resident's duties in the wards at any time. It was observed that after a man has graduated in medicine, served his internship and obtained his license to practice, he has become an important cog in the hospital machinery, and he, himself, sometimes thinks that his clinical services are far too important to "waste" time in laboratory work. Instances were cited where the resident was eager to improve his scientific background and came regularly to the laboratory with the best of intentions only to be called back almost immediately to the wards. Other examples were mentioned where residents felt that laboratory work should not be undertaken until all clinical duties were completed for the day, which brought the resident to the laboratory at times when supervised instruction was inconvenient, or impossible. The feeling at the meeting was very definitely in favor of regular, fixed, uninterrupted laboratory periods.

At another conference called to plan a more workable program for a group of related hospitals, one of the surgeons said that his idea would be for the resident to attend the operation on a given patient, take the specimen to the laboratory, describe it under supervision and finally have the finished section demonstrated by the pathologist or assistant pathologist. Such a plan might be ideal if a trained pathologist or an assistant were available for each resident in training, but that unfortunately is not the case, and the plan is otherwise impractical in the average hospital.

It would seem, therefore, that the first essential in graduate medical laboratory training is to reach an agreement concerning the time to be assigned to the laboratory, and that it be understood that the resident is not to be interrupted while on laboratory duty. The next most important element for success is cooperation between the clinical head of the specialty, the hospital management and the laboratory director. All must want to participate in the training program and be willing to make adjustments to maintain it. In no instance will it further the cause of the laboratory to have the clinician belittle the importance of laboratory procedures. The laboratory quarters should be attractive and easily accessible with a place for the resident to work. There should be plenty of autopsy and surgical materials and good records and files of past cases to draw from. There need not be enough current material for instruction in a given specialty if there is a good backlog of illustrative cases on record. It should not be forgotten that the greater part of any type of special pathology is general pathology applied to a given region or system. Once the resident has been assigned to the laboratory for a fixed time, he should participate in at least five autopsies on general cases, attend all gross description of surgical specimens and diagnostic sessions during his laboratory service, spend sufficient time in chemistry to be able to perform and interpret chemical tests germane

to his specialty and examine infected cases bacteriologically. He should have cases assigned to him for complete study including bacterial, chemical and pathologic tests. Finally, he should be required to write up a group of similar cases in order to learn how to prepare an article for publication. If possible, he should get his graduate training in anatomy and supplementary lectures in physiology at a medical school and reserve his time in the hospital laboratory for practical application in his studies.

Certification by Specialty Boards is now required by many hospitals as an essential to staff membership. The demand for resident training can no longer be met by medical centers alone and a greater number of general hospitals will be brought into the program. It is, therefore, important that a more uniform and concrete program of laboratory instruction be generally considered.

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BOOK REVIEWS

Microbial Antagonisms and Antibiotic Substances, Revised Edition. By SELMAN A. WAKSMAN, Professor of Microbiology, Rutgers University; Microbiologist, New Jersey Agricultural Experiment Station. 415 pp., 34 figs., 52 tables. \$5.00. New York: The Commonwealth Fund, 1947.

The second edition, like the first, is a compendium of knowledge concerning bacterial interrelationships, their antagonisms and certain metabolic products which have been studied for their possible usefulness in the control of infections. In this revised edition the author has assembled much new material that has appeared in the bacteriologic literature since the first edition was published in 1944. Abstracts from approximately 300 new references have been added, bringing the total of references to publications from all parts of the world to 1053. These are carefully documented in the text.

Obviously, a volume of 415 pages cannot embody more than mere mention of the observations already made in this field, but the author's selection of pertinent information should prove not only useful, but stimulating, to the microbiologist and chemist engaged in research. Regarding the accomplishments achieved and the outlook for the future the author states: "It was soon recognized that one is not dealing here with only three or more types of chemical compounds capable of destroying various pathogenic bacteria and fungi, but that a new field of science bordering on microbiology, chemistry, pharmacology, pathology and chemotherapy was being opened that was bound to result in many practical applications. The fact that many of these agents, including penicillin, are produced by several different organisms and, further, the fact that many of the compounds are formed in several chemical modifications open to the chemist new fields for the synthesis of types of compounds heretofore unknown, and point out to the medical world new ways of combating infections and epidemics."

The pathologist will also find this book stimulating even though it contains only suggestions for some procedures that may ultimately prove useful in his field of activity.

Detroit

JOSEPH A. KASPER

The Relation of Diseases in the Lower Animals to Human Welfare. By WILLIAM A. HAGAN, HERALD R. COX, WILLIAM H. FELDMAN, I. FOREST HUDDLESON, HAROLD N. JOHNSON, RAYMOND A. KELSER, JOSEPH V. KLAUDER, KARL F. MEYER, C. D. STEIN AND WILLARD H. WRIGHT. 223 pp., 3 figs., 11 plates, 21 tables. New York: The New York Academy of Sciences, 1947.

This is a series of papers presented by eminent authorities at a conference held by the New York Academy of Sciences. In the introduction, Hagan discusses the economic aspects of animal diseases on human welfare, showing that the cost of such diseases has a direct relation to the cost of living. The control of animal diseases is compared with methods for the control of human diseases in an interesting and pertinent manner.

The diseases discussed by the other authors include rabies, equine encephalomyelitis, psittacosis, brucellosis, plague, tuberculosis, anthrax, swine erysipelas and animal parasites. Each chapter includes the etiology and epidemiology of the disease with methods of control in both man and animals. To several of the chapters is appended the discussion which followed the presentation. Illustrations, maps and tables are numerous. Of particular interest is the table listing animal parasites found in lower animals and man, showing natural hosts, the locality where found and the frequency in man.

The material covered is complete and authentic. The pamphlet is recommended to all who may be interested in the subject.

Chicago

THOMAS G. HULL

The Diagnosis and Treatment of Diarrheal Diseases. By WILLIAM Z. FRADKIN, A.M., M.D., Assistant Attending Gastroenterologist and Associate Bacteriologist, Colitis Division, Department of Laboratories, Jewish Hospital of Brooklyn. 254 pp., 114 figs., 10 tables. \$6.00. New York: Grune & Stratton, Inc., 1947.

This volume is primarily designed for the general practitioner. The first section, "General Considerations" and the last, "Diarrheas of Infants and Children", begin with the analysis of the high morbidity and mortality of such diseases. The chapters on the anatomy, physiology and pathology of the intestinal tract are well written and strongly condensed. Sigmoidoscopy, diagnostic procedures and aids are described with great practical sense. The second part of the book, "Specific Diarrheal Diseases", contains valuable information resulting from the author's long personal experience. This is apparent in the chapters on amebiasis, bacillary dysentery, streptococcic diarrhea, chronic ulcerative colitis, diverticulosis etc., in which modifications and improvements of the standard therapeutic methods are presented. Controversial subjects, such as chronic ulcerative colitis, allergic diarrheas and psychogenic diarrhea are handled with tact and in a very intelligible manner.

The bacteriologic classification of salmonellae and shigellae, the contradictions among the descriptions and illustrations of some protozoa, many mistakes in the geographic distribution of diarrheal diseases and the inadequate descriptions of tropical diarrheas are of little importance to the American general practitioner, but constitute a definite shortcoming of the book from the point of view of the specialist.

Most of the numerous illustrations are excellent and very helpful.

Chicago

O. FELSENFELD

Recent Advances in Endocrinology. Ed. 6. A. T. CAMERON, C.M.G., M.A., D.Sc., F.R.I.C., F.R.S.C., Professor of Biochemistry, Faculty of Medicine, University of Manitoba, Biochemist, Winnipeg General Hospital. 443 pp., 74 figs., 3 plates. \$6.00. Philadelphia: The Blakiston Company, 1947.

This book brings together the pertinent facts in endocrinology in a concise manner. These facts are obtained by analyzing the current literature and selecting the material from outstanding articles in each field. The author has performed an admirable job in the selection of the material; almost always he has picked the original, as well as the major supporting articles, in each subject. There is room for expansion of this book, and in that way inclusion of more of the current developments.

San Francisco

GERSON R. BISKIND

Histopathology of the Ear, Nose and Throat. By ANDREW A. EGGSTON, B.S., M.D., Director of Laboratories Manhattan Eye, Ear and Throat Hospital, Clinical Professor of Pathology (Postgraduate Division) New York University Medical College, Director of Laboratories of the Mount Vernon Hospital, Pathologist Harlem Eye and Ear Hospital; and DOROTHY WOLFF, A.B., M.A., Ph.D., Research Investigator, Endaural Hospital, New York City, Fellow in Research, Harvard Medical School. 1080 pp., 505 figs., 28 color plates, 9 tables. \$18.00. Baltimore: The Williams & Wilkins Company, 1947.

This book contains carefully written chapters on comparative anatomy, embryology, histology and physiology. About one-half of the space is devoted to the ear, the remainder being apportioned to the nose, the pharynx and larynx.

The following criticisms of the work may be made. It is not complete, as the authors themselves admit. Some subjects are dealt with summarily. Perhaps too much space is given to theoretical considerations and not quite enough to microscopic descriptions of lesions. While the book is generously illustrated by 505 figures in black and white and 28 plates in color, the general attractiveness of the volume would have been enhanced by omission of those illustrations and color plates which lack good technical quality or teaching value, and by trimming of many others. A minor point of criticism concerns many of the drawings whose excellence is somewhat impaired by lettering that lacks the "professional touch".

In general, this is a useful reference book and can be recommended.

TECHNICAL SECTION

TECHNIC AND IDENTIFICATION OF FUNGI OF MEDICAL INTEREST*

EDWARD D. DELAMATER, M.D.

From the Section on Bacteriology, Mayo Clinic, Rochester, Minnesota

The purpose of this report is to emphasize certain laboratory aspects of the diagnosis of the various mycoses, and to present in abbreviated pictorial form the points in the diagnosis of each which deserve especial attention. The point of view presented is that of the laboratory worker who is presented with a specimen from which he is expected, by some means, to extract an etiologic agent; in this case, mycotic.

This general subject has been considered, with somewhat different emphasis, in several papers and texts which should be available for ready reference to those working in medical mycology or closely allied fields, such as clinical bacteriology.^{2-4, 6, 8-12} The numerous papers dealing with special aspects or problems within the field are readily obtainable from these source books, and except for special instances need not be repeated here. The author has drawn largely from his own experience in the preparation of this paper.

GENERAL TECHNIQUES

In general, the methods used in the laboratory diagnosis of various mycoses are simple and direct. Recognition of the well-established pathogens rests, for the most part, upon recognition of their morphologic characteristics, either in the pathologic specimen, or in culture, or in both. The simple procedures given here are directed to that end.

No effort will be made to discuss the histopathologic aspects of the diseases considered. It suffices to say that diagnosis cannot be made on the basis of the cellular response alone. Demonstration of the causative fungus is essential.

COLLECTION OF MATERIAL

Actual collection of material, whether it is sputum, pus, specimens for biopsy or other examination, usually rests with the clinician. It is, however, important to realize the need for adequate material with which to work; frequently it is necessary to make this need known. Ordinarily, there is little excuse for inadequate material, especially if diagnosis rests upon the efforts made in the laboratory. Constant reiteration of the need for properly collected specimens of sufficient amount to permit adequate study should be part of the activity of every laboratory worker. It is better that the laboratory be given the privilege of disposing of excess quantities rather than to receive a cubic centimeter or two of a liter specimen, the remainder of which has been disposed of from the bedside.

* Presented at the Twenty-Sixth Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 29, 1947. Received for publication, October 29, 1947.

The types of material in which mycosis should be suspected and the most common organism or organisms to be sought are given in Table 1.

In general, it may be said that specimens obtained by swabs are inadequate. Swabbings of the mouth and vagina may, however, be useful for the direct culture of *Candida* (*Monilia*) *albicans*.

DIRECT OBSERVATIONS

Both macroscopic and microscopic direct observations of material received should be routinely made. Macroscopic observation may lead to suspicion of the presence of actinomycetic granules in both sputum and exudates. The presence of micro-abscesses or miliary abscesses may lead to suspicion of blastomycosis or other mycoses in material for biopsy.

Microscopically, direct observation should be carried out in two ways. First, suspected material should be mounted in a drop of a 10 or 15 per cent solution of sodium hydroxide, warmed briefly, pressed out under a cover slip and studied microscopically for the presence of hyphal filaments, budding cells or actinomycetic granules. The gross presence of granules must be verified by microscopic observation of both stained and unstained preparations.

Second, smears should be stained. Both Gram's stain and Wright's stain are useful. The presence of cells suspected or observed in potassium hydroxide preparations frequently can be established and recognized by this means. Water mounts of material also may be stained with methylene blue. This procedure may be found to give an answer when other methods fail. As a rule, for general diagnostic work, iron-alum hematoxylin preparations of sectioned material give results that are as good as, or better than, those obtained by any other method. Specific measures applicable for each case considered will be emphasized as these are taken up.

CULTURAL METHODS

With certain exceptions, such as *Rhinosporidium* (if this be a mycosis), the pathogenic fungi can be grown readily on various mediums. It should be emphasized, however, that different fungi, like different bacteria, have different requirements for growth. This is mentioned because the idea has developed that Sabouraud's medium, composed of peptone and maltose or dextrose with a pH of approximately 5, is the medium of choice on which to grow all fungi. This is not the case. Sabouraud's medium was specifically developed for use with the dermatophytes; it has a relatively low pH so that the growth of bacteria will be inhibited. Some fungi grow poorly or not at all on this medium. Other mediums must, therefore, be used. The following mediums are recommended for the purposes indicated.

Sabouraud's medium: for primary isolation of dermatophytes and *Monilia* (*Candida*).

Cornmeal extract agar: for differentiation of yeasts and cryptococci from the medically important species of *Monilia* (*Candida*), and for specific recognition of *C. (Monilia) albicans*.

Dextrose nutrient agar and blood agar, to which 25 units per cc. each of penicillin and streptomycin has been added:¹³ for primary isolation of such organisms as *Histoplasma*, *Coccidioides* and *Blastomyces*. The antibiotic agents reduce bacterial contamination.

Dextrose nutrient agar and blood agar, without penicillin and streptomycin: for the culture of *Nocardia*.

Thioglycollate broth: for primary isolation of both *Actinomyces* and *Nocardia*.

TABLE 1

MOST USUAL FUNGOUS ORGANISMS TO BE LOOKED FOR IN SPECIMENS FOR EXAMINATION

SPECIMEN	ORGANISM
Hair	Dermatophytes, <i>Piedraia</i>
Scrapings from skin or nails	Dermatophytes, <i>Candida</i> (<i>Monilia</i>) <i>albicans</i> , <i>Malassezia furfur</i>
Pus from micro-abscesses and larger abscesses	<i>Blastomyces</i> , <i>Coccidioides</i> , <i>Actinomyces</i> , <i>Nocardia asteroides</i> , <i>Cryptococcus</i>
Pus and exudate from sinus tracts	<i>Blastomyces</i> , <i>Coccidioides</i> , <i>Cryptococcus</i> , <i>Histoplasma</i> , <i>Actinomyces</i> , <i>Nocardia</i> , various organisms found in Madura foot (such as <i>Monosporium</i>)
Blood	<i>Histoplasma</i> (other deep mycoses?)
Sternal aspirations	<i>Histoplasma</i> (other deep mycoses?)
Urine	<i>Blastomyces</i> , <i>Coccidioides</i> , <i>Cryptococcus</i> , <i>Histoplasma</i> , <i>C. albicans</i>
Stool	<i>C. albicans</i> , <i>Histoplasma</i>
Sputum	<i>C. albicans</i> , <i>Coccidioides</i> , <i>Blastomyces</i> , <i>Histoplasma</i> , <i>Cryptococcus</i> , <i>Actinomyces</i>
Exudates from body cavities	<i>Blastomyces</i> , <i>Coccidioides</i> , <i>Histoplasma</i> , <i>Cryptococcus</i> , <i>Actinomyces</i> , <i>Nocardia</i>
Cerebrospinal fluid	<i>Cryptococcus</i> , <i>Blastomyces</i> , <i>Histoplasma</i> , <i>Coccidioides</i> , <i>Actinomyces</i> , <i>Nocardia</i> , <i>C. albicans</i>
Scrapings from ulcers	<i>Histoplasma</i> , <i>C. albicans</i> , <i>Blastomyces</i> , <i>Coccidioides</i>
Nasal polyps	<i>Rhinosporidium</i>
Vaginal washings and swabs	<i>C. albicans</i>

The formulas for these mediums follow.

Sabouraud's agar

Distilled water.....	1000 cc.
Agar.....	15 Gm.
Peptone (Fairchild's).....	10 Gm.
Dextrose (chemically pure).....	40 Gm.

Adjust to pH 5.5, tube and sterilize

Cornmeal extract agar

Cornmeal.....	62.5 Gm.
Tap water.....	1500 cc.

Let stand in water bath at 57 C. for one hour.
Filter through paper. Make up to 1500 cc. volume.
Boil to dissolve.

Agar..... 19 Gm.

Tube and sterilize

Dextrose nutrient agar

Distilled water.....	1000 cc.
Peptone (Baeto).....	10 Gm.
Sodium chloride.....	5 Gm.
Beef extract.....	3 Gm.
Dextrose.....	5 Gm.
Agar	15 Gm.
Sodium hydroxide 1.0 normal.....	1 cc.

Tube and sterilize

Blood agar: routine

Thioglycollate medium: Difeo

The concurrent growth of bacteria in cultures set up for fungi frequently causes great difficulty, both in the isolation and in the recognition of pathogenic forms. This is especially true in view of the fact that cultures of fungi should be held a minimum of three weeks before they are discarded. Use of Thompson's penicillin-streptomycin medium circumvents some of these difficulties.¹³ Many of the fungous pathogens are slow to appear in culture, and grow much more slowly than bacteria. Concurrent growth of saprophytic fungi may cause even more difficulty, and for these we have no inhibitors. Cultures for the fungi which cause systemic mycoses should be incubated both at 25 to 30 C. and at 37 C. Cultures for the superficial fungi should be grown at 25 to 30 C., preferably in an incubator at constant temperature.

Certain organisms, notably *Blastomyces dermatitidis*, *Paracoccidioides* (*Blastomyces*) *brasiliensis* and *Histoplasma capsulatum*, grow in two phases. They are filamentous at 30 C. and below, and yeastlike at 37 C. Such differences in growth at different temperatures are useful diagnostically.

USE OF LABORATORY ANIMALS

The mouse probably is the most useful laboratory animal for study of the mycoses. It is susceptible to inoculation with *B. dermatitidis*, *P. brasiliensis*, *Coccidioides immitis*, *Sporotrichum schenckii*, *Cryptococcus hominis*, *H. capsulatum* and others, provided dosage is sufficiently large. The disease produced and the form of the organism recovered from the lesions are frequently useful in recognition of the various pathogens.

In at least two instances namely, *Coccidioides* and *Sporotrichum*, and probably in others, it is felt that the use of inoculation of animals is most useful in the identification of the fungus. In other instances, inoculation of animals confirms the

diagnosis made on the basis of culture and adds information concerning the virulence of the particular fungus involved.

In our experience, inoculation of mice also has been used as a means of primary isolation of a suspected fungus from contaminated clinical material, when cultures fail. Inoculation of animals has the added advantage, in the case of certain organisms, of probably being safer than the study of open cultures.

DISEASE-PRODUCING FUNGI

Dermatophytes. In dealing with material such as hair, scrapings of skin and nails from patients with ringworm diseases, the important point is to establish the presence or absence of a fungous pathogen. This can be done by direct examination of potassium hydroxide preparations. With experience, a pathogen usually is recognizable in tissue. In scrapings of skin, the organisms are either continuous filaments or filaments broken into chains of so-called arthrospores. Both conditions are to be seen in the accompanying photographs (Figs. 1a and 1b). The problem is to become sufficiently adept in recognition of these organisms as they appear in tissue so that confusion does not arise in respect to various artefacts which may occur. In scrapings of nails, the organisms are similar in configuration to those seen in skin. In hair, the organisms may take one or two positions. They may penetrate and burrow through the hair shaft (Fig. 1c) (endothrix), or they may form a sheath of spores about the hair shaft (Fig. 1d) (ectothrix). Certain organisms may combine these two methods of attack on the hair and show both characteristics (neo-ectothrix). Actual culture and identification of the dermatophytes is not essential to either the diagnosis or the treatment of the ringworm diseases.⁶ Their culture and recognition are more of academic interest.

Malassezia furfur (Tinea versicolor). This organism ordinarily is not grown in culture. It can be recognized easily in direct preparations made with methylene blue. Clusters of round budding cells and mycelial filaments take up the stain avidly, and are easily identified (Fig. 1c) in the scales.

Nocardia minutissima (erythrasma). This organism is rapidly identified in the same manner as is *M. furfur*. It occurs as minute bacillary forms, and ordinarily is better seen with an oil immersion lens.

C. albicans frequently is isolated from specimens of stool, sputum, scrapings of skin and nails and occasionally from other sources. Regardless of the sources from which it is obtained, it can be seen either as budding cells, which must be cultured for identification, or as both budding cells and filaments. When in this combined form, it usually can be recognized (Figs. 1f and 1g).

It grows readily on Sabouraud's agar, dextrose agar or blood agar. On these mediums it produces typically creamy, yeastlike colonies comparable to those of a yeast. The cells are typically budding yeastlike cells. In cornmeal agar they develop a characteristic morphology in which there are filaments with clusters of budding cells at the nodes. Terminal chlamydospores or resting cells also occur (Fig. 1h). The complicated procedures outlined by Conant and his associates are not necessary to recognition of this fungus.^{1, 6}

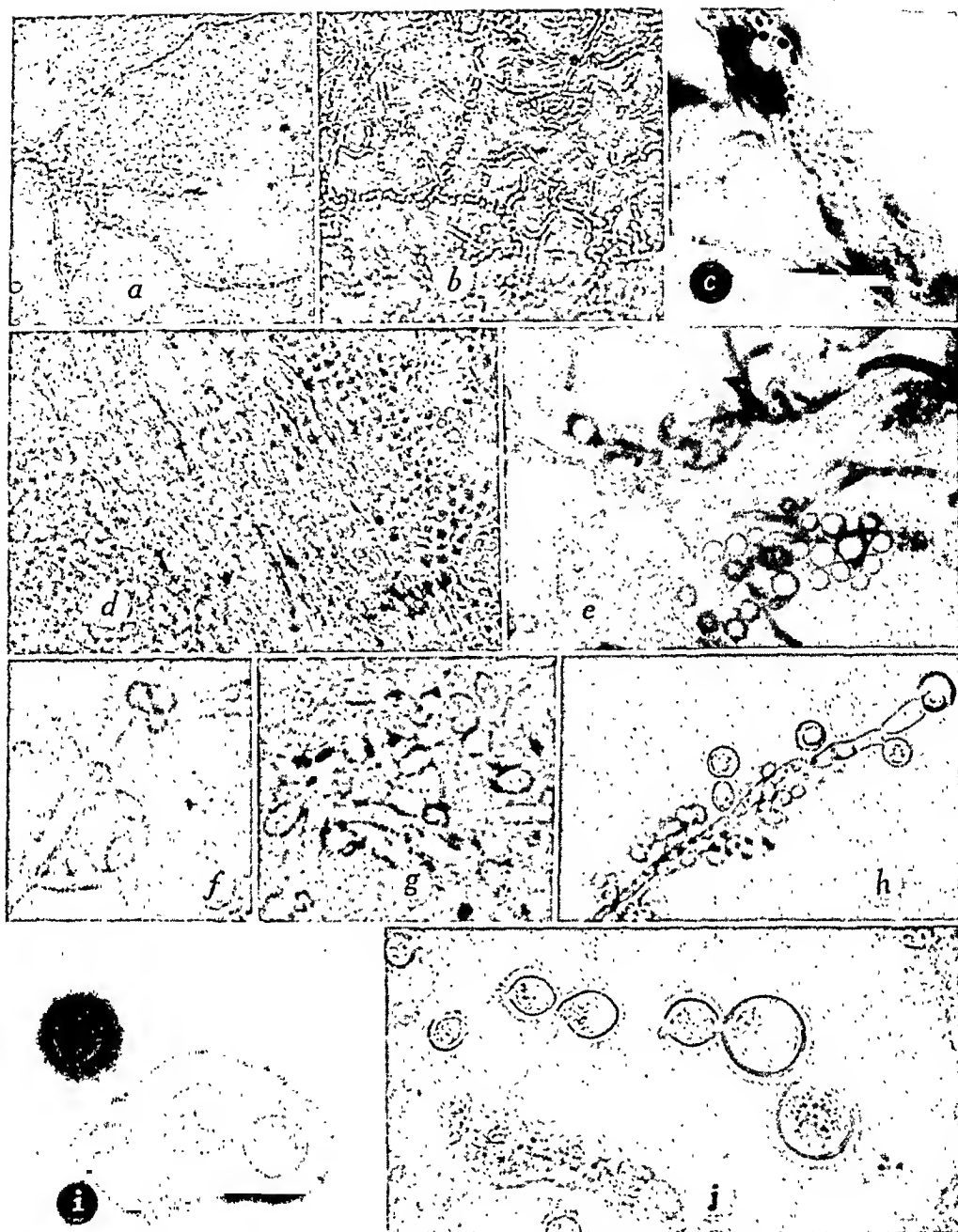


FIG. 1. *a. Trichophyton purpurcum*: unfragmented hyphal filaments in tissue; potassium hydroxide fresh preparation; *b. Trichophyton mentagrophytes*: hyphae fragmented into arthrospores in tissue; potassium hydroxide preparation; *c. Trichophyton sp.*: hyphae in "endothrix" position in hair shaft; note arthrospores; section for biopsy; iron-alum hematoxylin preparation; *d. Microsporum canis*: hyphae and arthrospores in "ectothrix" position forming sheath about hair shaft; *e. Malassezia furfur*: filaments and round bodies in scales; methylene blue preparation; *f. Candida albicans*: budding cells and filaments in tissue; potassium hydroxide preparation; *g. C. albicans*: budding cells and filaments in tissue; potassium hydroxide preparation; *h. C. albicans*: microscopic morphology in cornmeal extract-agar culture; note yeastlike cells, hyphae and chlamydospores; *i. Cryptococcus hominis*: microscopic morphology in culture; India ink preparation; note budding cells in clear capsular space surrounded by India ink; *j. C. hominis*: microscopic morphology in tissue; potassium hydroxide preparation; note budding cells in clear capsular space surrounded by tissue debris.

C. hominis (*Torula histolytica*) is to be looked for in cerebrospinal fluid, sputum and material from exudative processes. It can be visualized by direct examination as a budding cell, surrounded by a clear space or capsule (Figs. 1i and 1j). Diagnosis based on direct examination always should be verified by culture, with subsequent intracerebral inoculation of a mouse with the fungus isolated. In culture, the organism grows as a buff-colored or creamy fluid or gelatinous colony. The cells are typically surrounded by a gelatinous capsule which can be readily demonstrated in India ink preparations (Fig. 1i). (India ink is diluted 1:1 with water and some organisms are added with a platinum loop. A cover slip is placed upon the mount and sealed in place with petroleum jelly.)

B. dermatitidis is to be found in pus from micro-abscesses, material for biopsy, urine and sputum. In either stained or fresh mounts of tissue, it is to be recognized as a budding cell having a thick, double, refractile wall with a smooth surface (Figs. 2a and 2b). The bases or origins of the buds ordinarily are broad in contrast to *Cryptococcus*, in which the bases are narrow.

The fungus grows readily on dextrose or blood agar, more slowly on Sabouraud's agar. It has two growth phases in culture, depending on the temperature. At 30 C. and below *Blastomyces* grows as a filamentous plant and produces no budding cells. Colonies are white to buff, cottony or membranous. The fungus produces smooth-walled pyriform conidia borne singly on short or long stalks (Figs. 2c and 2d). These vary in size from 1 to 2 microns to 5 to 15 microns. The larger conidia may vary considerably in shape. At 37 C., on many mediums, the organism grows more slowly as a buff-colored, pasty or warty colony, the cells of which compare with those seen in tissue. The cells appear as round or oval, smooth-walled budding cells (Fig. 2e), or as more elongate cells (Fig. 2f) or, more commonly, as mixtures of the two types. Both types are typical of the yeastlike phase of growth of *Blastomyces*.

For verification of the diagnosis, a mouse should be inoculated either intravenously or intraperitoneally with the organism. In the mouse the organism produces a characteristic disease in which the budding cells can be found readily.

C. immitis is to be expected from the same types of pathologic material as *Blastomyces*. In either fresh mounts of tissue or in stained preparations this organism occurs in two forms. First, budding cells, very like those of *Blastomyces*, are to be found⁵ (Fig. 2g). Second, the characteristic form of the fungus is the much-emphasized spherule or sporangium (Fig. 2h). These structures may vary in size from 5 or 6 to 80 or 90 microns, and their walls are very thick and, frequently, spiny. The contained endospores may range in size from 1 or 2 microns to 5 or 6 microns. They are liberated by fracture of the wall of the spherule (Fig. 2h).

In culture, *Coccidioides* grows as a filamentous fungus which has no specific characteristics. It produces so-called racquet mycelia (Fig. 2i), which are typical of this fungus but which also occur in a wide variety of fungi. Colonies are white and cottony. The fungus does, however, produce arthrospores (chlamydospores) by fragmentation of the hyphal threads (Fig. 2j). Although these cells are characteristic of *Coccidioides*, they are not of diagnostic importance, since other fungi produce similar structures.

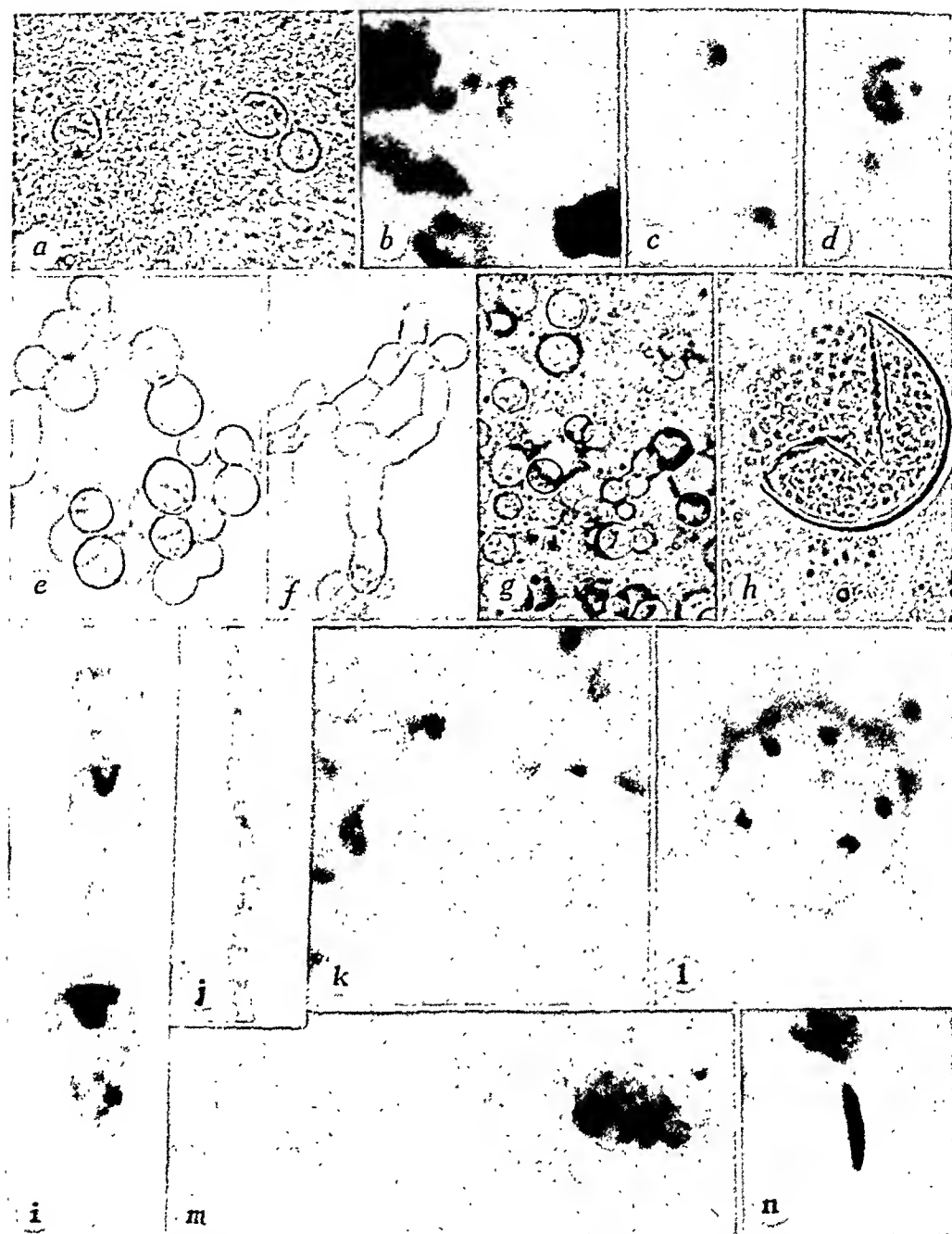


FIG. 2. *a.* *Blastomyces dermatitidis* in tissue: note budding cells; potassium hydroxide preparation of pus from micro-abscess; *b.* *B. dermatitidis* in tissue: DeLamater-Ulrich basic fuchsin-formaldehyde stain of section for biopsy; note cell wall of organism with protoplast containing four nuclei ($\times 3400$); *c.* *B. dermatitidis* in culture at 30 C.: pyriform conidia; iron-alum hematoxylin preparation ($\times 1700$); *d.* *B. dermatitidis* in culture at 30 C.: conidium ($\times 3400$); *e.* *B. dermatitidis* in culture at 37 C.: round budding cells; fresh preparation; *f.* *B. dermatitidis* in culture at 37 C.: abortive hyphal elements; fresh preparation; *g.* *Coccidioides immitis* in tissue: fresh preparation; note budding cells; *h.* *C. immitis* in tissue: typical spherule with enclosed endospores: spherule (sporangium) ruptured; fresh preparation; *i.* *C. immitis* in culture at 30 C.: racquet mycelium; iron-alum hematoxylin preparation; *j.* *C. immitis* in culture at 30 C.: arthrospores (ehlamydospores) formed by fragmentation of hyphae; iron-alum hematoxylin preparation; *k.* *Histoplasma capsulatum* in tissue: note numerous minute, oval, budding cells; iron-alum hematoxylin preparation of section of tissue; *l.* *H. capsulatum* in culture at 30 C.: characteristic tuberculate ehlamydospore; DeLamater-Ulrich basic fuchsin-formaldehyde stain, showing nuclei; *m.* *H. capsulatum* in culture at 30 C.: tuberculate ehlamydospore and immediate conidia; DeLamater-Ulrich basic fuchsin-formaldehyde preparation; *n.* *Sporotrichum schenckii* in tissue: characteristic cigar-shaped body; Gram stain of tissue-impression smear.

Intraperitoneal inoculation of mice with any fungus suspected of being *Coccidioides* should be routine, because the characteristic spherules, along with budding cells, are regularly produced in this animal and can be demonstrated readily by examination of direct preparations. Incidentally, it is much safer to use this procedure than to work with this fungus in culture.

H. capsulatum is to be expected in smears from ulcers situated about the nose, mouth, penis and anus as well as in sputum, sternal aspirations, stools and material for biopsy. In tissue it occurs as a small ovoid, budding cell, 2 by 3 microns, with what appears to be a narrow capsular space about it (Fig. 2k). It occurs free or in the cytoplasm of macrophages, where it produces a very characteristic picture. Diagnosis, especially on the basis of smears of ulcers, cannot always be made in the presence of such cells, however, particularly if other recognizably different organisms, such as *C. albicans*, also are present. *C. albicans* produces some minute budding cells which easily can be confused with the tissue phase of *Histoplasma* in smears.

Confirmation by culture is necessary. On dextrose or blood agar at 30 C., the organism grows as a white, fluffy colony. Growth is filamentous. Two types of spores are produced. The first is the so-called tuberculate or spiny-walled chlamydospore or resting cell which is characteristic of this fungus (Fig. 2l). These cells range in size from 5 to 20 microns or larger. The second type of spore is the small thin-walled, oval or pyriform conidium which is borne on short stalks along the hyphal filaments (Fig. 2m).

Under special conditions, at 37 C. in sealed tubes, the fungus can be induced to grow in the form of a minute bacteria-like colony in which only budding cells similar to those occurring in tissue are found.

S. schenckii is to be sought in pus or material for biopsy obtained from chancre-like lesions or abscesses developing along lymphatic channels, and occasionally from other types of pathologic specimens. It is extremely difficult to demonstrate this fungus by direct visual methods in stains and smears of tissue. When it is found, it occurs as minute, spindle-shaped or cigar-shaped cells 1 by 2 microns (Fig. 2n).

Culture of tissue, however, readily produces growth. Growth is filamentous. Colonies are light or dark, and frequently change color with continued cultivation. Single-celled conidia are formed in profusion, and are produced on specialized conidiophores (Figs. 3a and 3b) and as a collarette about a hyphal filament.

Mice or rats should be inoculated intraperitoneally with this fungus, which produces a characteristic infection in the peritoneum, scrotum, joints of the tail and frequently in other joints. Smears of animal tissue usually show numerous typical bacilliform cells (Fig. 2n).

Aspergillus fumigatus and other species of *Aspergillus* and *Penicillium* are to be found in material from nails, abscesses and from sputum. In tissue, both mycelia and typical conidiophores are to be found (Fig. 3c).

In culture, this and other related fungi grow rapidly on usual mediums, and are distinguished by their specific characteristics on Czapek's agar. Typical conidiophore heads are produced. The student is referred to Thom and Raper's *A Manual of the Aspergilli*¹² for differentiation.

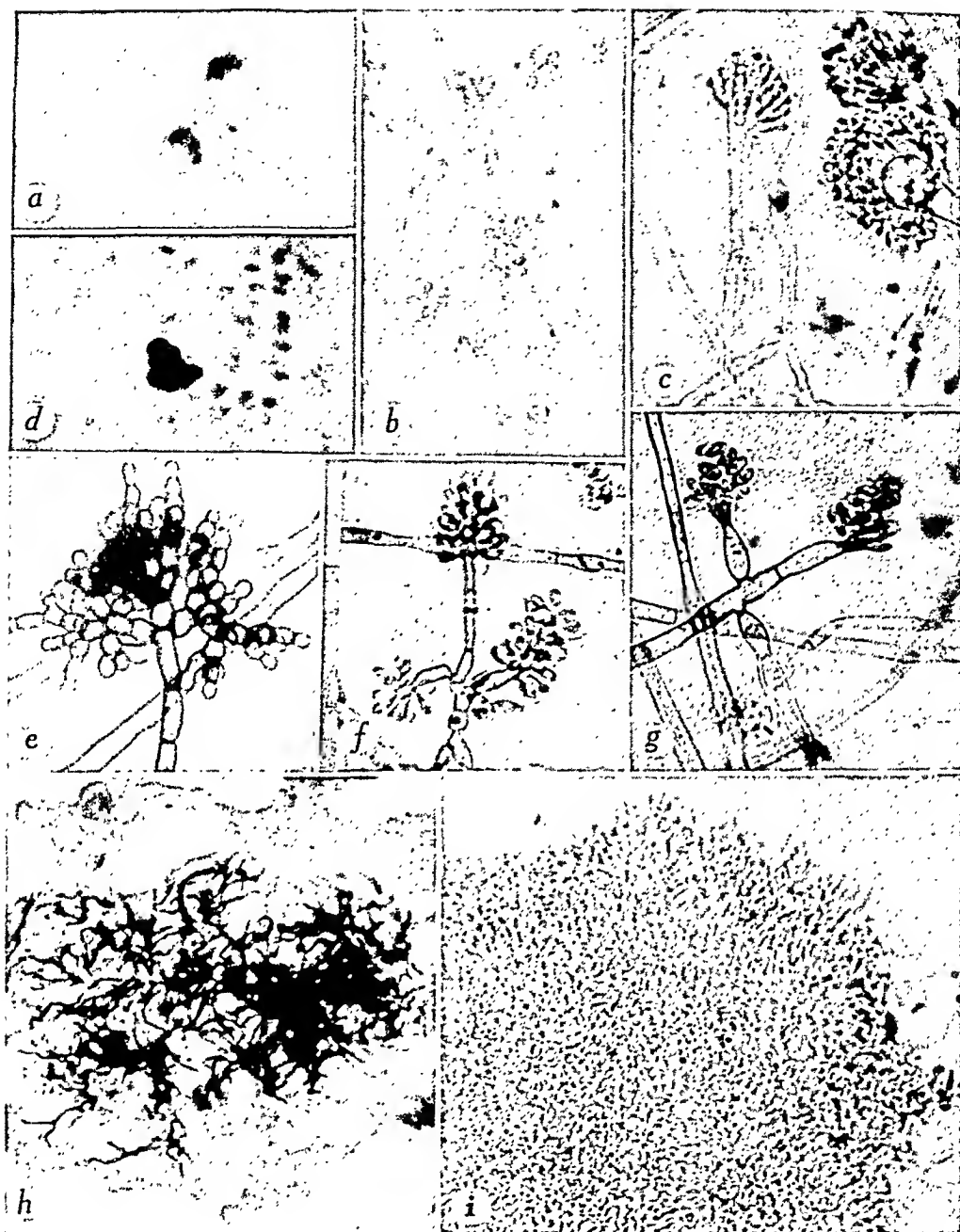


FIG. 3. *a.* *Sporotrichum schenkii* in culture at 30 C.: cluster of conidia-aerothea type sporulation; fresh preparation; *b.* *S. schenkii* in culture at 30 C.: hyphae and elusters of conidia; fresh preparation; *c.* *Aspergillus fumigatus* in culture: conidiophores and hyphae; similar struetures may be observed in tissue; fresh preparation; *d.* *Hormodendrum pedrosoi* in tissue: irregular, highly colored fungous body; iron-alum hematoxylin preparation; *e.* *H. pedrosoi*: Hormodendrum type sporulation; fresh preparation (Carrión); *f.* *H. pedrosoi*: Aerothea type sporulation; fresh preparation (Carrión); *g.* *H. pedrosoi*: Phialophthora type sporulation with phialide or euplike conidiophore; fresh preparation (Carrión); *h.* *Nocardia asteroides* in tissue: note minute branching filaments; iron-alum hematoxylin preparation; *i.* *Actinomyces bovis* in tissue: soft granules showing filamentous structure and lack of asteroid elubs; potassium hydroxide preparation.

Hormodendrum pedrosoi. This fungus is to be found in material taken for biopsy, from warty growths, particularly of the extremities. In direct examination of either fresh preparations or stained sections of the material, the fungus appears as a dark brownish or olivaceous, irregularly-shaped cluster of rather thick-walled cells (Fig. 3d).

The organism grows slowly on all the usual mediums at 30 C. as a darkly pigmented olive drab to blackish heaped-up filamentous colony. The microscopic morphologic aspects are complex. Three types of spore-bearing structures should be looked for: the *Hormodendrum* type (Fig. 3e), the *Acrotheca* type (Fig. 3f) and the *Phialophthora* type (Fig. 3g). All three types may not be present in the same culture at the same time, and they may vary widely in their relative number and occurrence.

Actinomyces bovis and *Nocardia asteroides*. These two organisms may be found in a wide variety of clinical material. They will be dealt with separately, because their morphologic characteristics as well as their cultivation differ markedly. Differentiation is important clinically because each is sensitive to a different drug.

N. asteroides. In tissue this fungus does not produce granules. It grows as a loose web of minute filaments or branching threads (Fig. 3h).

The organism can be grown upon blood agar or dextrose agar and in thioglycollate broth, where it grows in the uppermost one-half inch of the medium as round or irregular tufts. The microscopic appearance is very similar to what is seen in tissue. Recognition must be based upon microscopic evaluation, lack of sporulation, size of filaments and branching, and gram-positiveness. Inoculation of mice with this fungus may produce transitory disease with focal abscesses in the liver, kidney, lungs and brain.

A. bovis. This fungus occurs classically in tissue as a granule, or pinhead-sized or larger colony, composed of densely packed, closely entwined minute filaments. Typically, these colonies are surrounded by club-shaped radiate structures, but these need not occur (Fig. 3i). There is considerable question as to whether *A. bovis* produces granules in all cases. *A. bovis* is anaerobic or micro-aerophilic.

Culture is difficult, especially if the source material is contaminated. In thioglycollate broth the fungus develops in the depths as minute round or irregular colonies which must be identified as filamentous by means of the microscope. Filaments may fragment into bacillary forms. The student is referred to the work of Emmons for further elucidation of this complex subject.

Inoculation of animals with the organism is of no help.

A. bovis is sensitive to penicillin; *N. asteroides* is sensitive to the sulfonamide compounds. The use of penicillin in mediums as an inhibitor of contaminants, when penicillin-sensitive diseases are suspected, is thus impossible.

SUMMARY

The diagnostic features of several of the more important mycoses have been presented briefly.

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EFFECT OF pH ON STREPTOMYCIN ACTIVITY*

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Several studies on the effect of pH on the action of streptomycin have already been reported.^{1,4,6-8} In liquid mediums, an increase in the acidity of the medium has resulted in an appreciable and sometimes marked increase in the concentration of streptomycin required to inhibit the growth of the various bacteria that have been tested.^{1,6-8} This effect was noted both in highly nutritive broth and in less nutritive mediums,⁶ and also with both large and small inoculums.¹ In the assay of streptomycin by the agar diffusion method employing filter paper discs, the diameter of the clear zone of inhibition produced in the seeded agar by any given concentration of streptomycin increased with increases in the initial pH of the nutrient agar.⁴ Clinically, it also has been noted, at least in urinary tract infections, that the maintenance of an alkaline reaction in the urine during treatment with streptomycin resulted in the elimination of bacteria from the urine with considerably greater regularity than in patients whose urine was acid during streptomycin therapy, and that resistant strains appeared much more often in the latter.^{2,3}

Previous unpublished studies in this laboratory on the effect of pH on the action of streptomycin in liquid mediums yielded irregular results, particularly with *Pseudomonas aeruginosa* as the test organism. This was due to the frequent emergence of resistant strains even during the course of a single subculture in streptomycin-containing mediums, an unpredictable complication which suggested the desirability of examining this effect on isolated cells. With that in view, a quantitative study was made of the effect of pH on the action of streptomycin in an agar medium on one strain each of *Aerobacter aerogenes*, *Escherichia coli*, *Ps. aeruginosa* and *Proteus vulgaris*.

This was accomplished by pouring agar plates containing graded concentrations of streptomycin and relatively large numbers of organisms. The inhibitory action of streptomycin on susceptible cells could thus be observed without the organisms being overgrown and masked by any resistant variants that might be present originally or that might appear during growth. Such organisms would be distinguished as individual colonies in the agar. The agar pour-plate method also has at least two additional advantages: (1) Since inhibition is used as the end point, presumably only small amounts of bacterial metabolites are produced in the mediums when such inhibition takes place and it should not be necessary to provide extra buffer capacity. (2) The numbers of colonies growing in concentrations of streptomycin lower than those which cause complete inhi-

* Aided by a grant from the United States Public Health Service. Received for publication, November 3, 1947,

bition can be counted and thus provide additional information as to what happens in the range of incomplete inhibition.

MATERIALS

Nutrient agar. A stock was prepared from Heart Infusion Agar, Dehydrated, (Difco), pH 7.4. Stocks at three additional pH levels, roughly 8.0, 6.3 and 5.5, were prepared from this by the addition of appropriate amounts of sodium hydroxide or hydrochloric acid as needed, while the medium was liquid and prior to sterilization. The actual pH was checked immediately before each experiment.

Broth. The broth used was made from dehydrated brain heart infusion broth (Difco), pH $7.4 \pm$. This was used for growing and diluting the cultures and also for diluting the streptomycin solutions.

Streptomycin. A stock solution of streptomycin hydrochloride (Merck), containing 0.1 Gm. per ml. was prepared by dissolving the equivalent of 1.0 Gm. of the base in broth to a final volume of 10 ml. Working dilutions were prepared from this stock solution as needed. All streptomycin solutions were kept at 4 C. when not being used.

Organisms. Four strains were used, (1) *A. aerogenes*, (2) *E. coli*, (3) *Ps. aeruginosa* and (4) *P. vulgaris*.* Each of these strains had been extensively studied in this laboratory. They were inhibited by streptomycin in concentrations of 12.5, 25, 12.5 and 50 μ Gm. per ml., respectively, as determined by the serial dilution method in broth.² All had produced resistant variants *in vitro*.⁵ They had been isolated originally from patients with urinary tract infections prior to treatment with streptomycin. Resistant strains of the corresponding organisms had appeared in the urine of the patients from whom the first three strains were obtained, while the *Proteus* infection cleared up rapidly during the course of the streptomycin therapy and resistant strains of that organism were not isolated directly from the patient.

Indicators. Phenol red, 0.04 per cent solution; bromcresol purple, 0.04 per cent.

METHODS

Cultures of the four organisms were streaked on agar plates. A representative colony was selected from each plate and transferred to broth which was then incubated for approximately eighteen hours and served as the seed culture for the experiment. Four rows of plates, corresponding to four levels of pH were then arranged for each organism. The number of plates in each row was arranged to correspond roughly to the range of streptomycin concentration within which it was anticipated that inhibition would occur, depending on the known sensitivity of the organism. After working dilutions of streptomycin were made up in twofold dilution series, individual plates were prepared by adding 1.0 ml. of the appropriate streptomycin solution, 0.1 ml. of the seed culture and

* These strains represent numbers 3, 8, 7 and 9, respectively, in a study previously reported on the development of resistance to streptomycin *in vitro*.⁵

8.9 ml. of melted agar, making the final volume 10 ml. The streptomycin was thus diluted tenfold and provision was made for this in making the working dilutions. Care was taken not to allow mixture of the inoculum and the streptomycin until after the addition of the agar, at which time the materials were mixed carefully in order to insure a thorough distribution of bacteria and streptomycin. The agar was allowed to harden at room temperature after which the plates were incubated at 37 C. for about twenty-four hours and the total number of colonies counted.

Control plates poured for each row consisted of: (1) Agar and streptomycin but no organisms. This served to check the pH of the mixture before and after incubation. (2) Streptomycin-free agar inoculated with 0.1 ml. of a 10^{-6} dilution of the culture used above. The latter provided information as to the colony count of the culture. In addition, a comparison of the number of colonies derived from the same inoculum growing at different pH levels, would be expected to show any significant inhibitory effect of the pH alone.

The initial pH of the agar was determined colorimetrically using phenol red and bromeresol purple as indicators. Streptomycin in the quantities used did not affect the pH appreciably. The pH of the material in a plate was determined by placing a drop of indicator on the surface of the agar. After a few minutes during which the dye became fixed to the solid surface, the plate was arranged in front of a comparator block in such a manner that the tinted portion was in front of the control tubes while an untinted portion was in front of the holes for the standard tubes. Comparisons with standard pH sets could be made with relative ease. Determinations of pH were made in this manner on streptomycin free plates before and after incubation and on all other plates immediately after the colony counts had been made.

RESULTS

The numbers of colonies that developed in the various plates are shown in Table 1. The decline in the inhibitory action of the streptomycin with each decrease in the initial pH of the medium is clearly demonstrated for each of the four organisms. This is also shown in Table 2 which lists the minimum inhibiting concentration of streptomycin (as judged by the failure of any recognizable colonies to develop) for each of the organisms at the different pH levels. In the case of *E. coli* the concentration required for complete inhibition was twenty times greater, and for *A. aerogenes* it was eighty times greater, at pH 5.8 than at pH 7.9, and for *Ps. aeruginosa* and *P. vulgaris* the minimum inhibiting concentration was forty times greater at pH 5.8 than at pH 8.0.

It is also noted in Table 1 that the end points were not sharp but there were always some concentrations of streptomycin in which there was partial inhibition. This was equally true for each of the organisms and at each of the pH levels.

The changes that occurred in the pH of the medium in the different plates are also of interest. In those plates in which little or no growth took place the pH remained essentially unchanged. Where there was slight to moderate

TABLE 1
EFFECT OF pH ON THE INHIBITORY ACTION OF STREPTOMYCIN

ORGANISM	INITIAL pH*	FINAL CONCENTRATION OF STREPTOMYCIN, MICROGRAMS PER ML. OF AGAR										0.78	1.56	3.12	6.25	12.5	25	50	100	250	500	1000	2000	4000†	0.1
		0	0.1	0.2	0.3	0.5	0.7	1.0	1.5	2.0	3.0														
<i>A. aerogenes</i>	7.9											++	362	0	0	-0	0**								150§**
	7.2											++	++	708	5	7.8	7.8								8.3
	6.3											++	++	++	7.2	7.2	3	0	0						1.49
	5.8											++	++	++	++	108	6.5	6.4	6.4						8.2
	5.8											++	++	++	++	++	++	159	3	0	0				159
<i>E. coli</i>	7.9														7.7	7.45	6.9	6.2	5.8						140
	7.2																								8.2
	6.3																								120
	5.8																								8.1
	5.8																								110
<i>Ps. aeruginosa</i>	8.0																								123
	7.2																								8.1
	6.3																								8.2
	5.8																								8.1
	5.8																								200
<i>P. vulgaris</i>	8.0																								8.4
	7.2																								243
	6.3																								8.3
	5.8																								228
	5.8																								8.4

* , pH of agar before incubation. †, Inoculum 0.1 ml. of an eighteen hour culture in plates containing streptomycin. ‡, Inoculum in streptomycin-free plates was 0.1 ml. of a 10⁻⁶ dilution of the culture. **, Upper number is the colony count (represented by plusses when too many to count), the lower number is the final pH of the agar.

growth, there was occasionally a slight drop in pH from original high levels. In the plates showing heavy growth and in the control plates which contained no streptomycin, the final pH was about 8.0 or higher, irrespective of the initial pH. The final pH was somewhat higher in the heavily grown plates with *Ps. aeruginosa* and *P. vulgaris* than was the case with the cultures of *A. aerogenes* and *E. coli*.

TABLE 2

STREPTOMYCIN SENSITIVITY OF FOUR STRAINS AS DETERMINED ON AGAR POUR PLATES AT FOUR pH LEVELS

ORGANISM	INITIAL* pH	MINIMUM INHIBITING CONCENTRATION OF STREPTOMYCIN†	
		Gm. per ml.	Comparison with result at pH 8.0
<i>A. aerogenes</i>	7.9	3.12	1
	7.2	12.5	4
	6.3	100	32
	5.8	250	80
<i>E. coli</i>	7.9	25	1
	7.2	100	4
	6.3	250	10
	5.8	500	20
<i>Ps. aeruginosa</i>	8.0	100	1
	7.2	250	2.5
	6.3	2000	20
	5.8	4000	40
<i>P. vulgaris</i>	8.0	50	1
	7.2	250	5
	6.3	1000	20
	5.8	2000	40

* The final pH of the agar in the plates showing complete inhibition was essentially the same.

† Complete inhibition as judged by failure of any colonies to develop.

DISCUSSION

The present studies confirm previous observations that an increase in the acidity of the culture medium reduces the inhibitory action of streptomycin. The findings are also in accord with the assumption that any given culture consists of organisms of varying sensitivity and resistance to streptomycin. An increase in the pH of the medium at any given concentration of streptomycin therefore results in the inhibition of a larger number of organisms and the result is equivalent to that obtained at the same pH with a higher concentration of streptomycin.

In the case of the present cultures, when growth was essentially uninhibited the medium became alkaline irrespective of the initial pH. The failure of the

streptomycin to inhibit that growth may depend on the fact that the numbers of organisms present by the time the medium became alkaline may already have increased to a point where they were not affected at the concentration of streptomycin present, even with the enhanced inhibitory effect at the higher pH level. Presumably, the streptomycin was not destroyed during the period of incubation. Furthermore, the initial acidity probably did not inactivate the streptomycin, since it has been shown that the full activity of streptomycin in acid solution can be restored by readjusting the pH upward.⁸

SUMMARY

The effect of pH on the inhibitory action of streptomycin on four different gram-negative bacilli was studied in deep agar cultures. The findings of previous workers were confirmed. There was a twenty to eightyfold reduction in the activity of streptomycin at pH 5.8 as compared with a pH of about 8.0.

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CARDIOLIPIN KOLMER ANTIGEN IN TESTING ICTERIC SYPHILITIC SERUMS*

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In Kolmer complement-fixation tests, discrepancies were found between the results obtained when testing icteric, low-titered syphilitic serums with standard Kolmer antigen and with cardiolipin-lecithin Kolmer antigen. The antigen and amboceptor for this study were obtained from Dr. John A. Kolmer, and the cardiolipin-lecithin antigen was furnished by Dr. J. F. Mahoney, Venereal Disease Research Laboratory, Marine Hospital, Staten Island, N. Y.

Parallel complement-fixation tests with the two antigens in tests of five icteric known syphilitic serums submitted for routine examination resulted in the reactions shown in Table 1.

It appeared from the above tests that the maximum positive dilution still giving a positive test in icteric, syphilitic serums was higher in the cardiolipin-lecithin antigen than in the regular antigen used in the complement-fixation test and that the use of the former antigen might have the advantage of eliminating false-negative reports. The possibility of the existence of a direct relationship between the number of units of the icterus index and the dilution in the cardiolipin-lecithin Kolmer test at which a regular Kolmer antigen might fail to reveal evidence of syphilis was considered.

Bile from a surgically removed gallbladder was used in the preliminary tests and the above findings were confirmed. Later, icteric serum was substituted for the bile. Controls without icteric serums were used in sufficient numbers to insure valid results. Pooled negative serums, pooled positive serums and negative icteric serums were diluted to the desired Kolmer units. Controls contained the same amount of pooled positive serum, with pooled negative serum replacing the icteric serum. More than 500 such icteric, positive serums and their controls, were tested with both cardiolipin-lecithin Kolmer antigen and the regular Kolmer antigen, and representative readings with the two antigens and controls are shown in Table 2.

It was found that the cardiolipin-lecithin antigen was more sensitive than the regular Kolmer antigen, and that a higher icteric index was necessary to prevent fixation of complement when the cardiolipin antigen was used.

A positive serum containing two cardiolipin Kolmer units and an icterus index of 34 units failed to show any complement-fixation when the regular Kolmer antigen was used, while the cardiolipin-lecithin Kolmer antigen units were only reduced.

In general, the higher the number of cardiolipin-lecithin Kolmer units, the greater the icterus necessary to block complement-fixation; while with an increasing number of regular Kolmer units the icterus index necessary for blocking

* Received for publication, October 22, 1947.

was less predictable. Complement-fixation was completely blocked in the regular Kolmer antigen before any effect was noticed in the fixation of complement in tests in which the cardiolipin-lecithin antigen was used. It was possible

TABLE 1

COMPARISON OF RESULTS OF COMPLEMENT-FIXATION TESTS ON FIVE ICTERIC SYPHILITIC SERUMS USING STANDARD KOLMER AND CARDIOLIPIN-LECITHIN KOLMER ANTIGENS

SERUM NUMBER	KOLMER ANTIGEN USED		ICTERUS INDEX
	Cardiolipin-Lecithin	Standard	
			<i>units</i>
1	44	--	Not determined
2	43	+±	29
3	44	+±	Undetermined (Bile in urine)
4	2-	--	Not determined
5	+ -	--	Not determined

TABLE 2

RESULTS OF COMPLEMENT-FIXATION TESTS OF 16 REPRESENTATIVE SYPHILITIC SERUMS USING STANDARD KOLMER AND CARDIOLIPIN-LECITHIN KOLMER ANTIGENS (a) WHEN SERUMS WERE DILUTED WITH NORMAL SERUM AND (b) WHEN SERUMS WERE DILUTED WITH ICTERIC SERUM

SERUM NUMBER	SERUM DILUTED WITH NORMAL SERUM		Icterus Index	SERUM DILUTED WITH ICTERIC SERUM	
	Standard Kolmer Antigen	Cardiolipin Kolmer Antigen		Standard Kolmer Antigen	Cardiolipin Kolmer Antigen
			<i>units</i>		
1	- - - - -	2 - - - - -	34	- - - - -	+ - - - -
2	- - - - -	2 - - - - -	34.4	- - - - -	+ - - - -
3	2 - - - - -	3 + - - - -	49.3	- - - - -	+ - - - -
4	- - - - -	4 - - - - -	27.6	- - - - -	+ - - - -
5	+ - - - -	2 - - - - -	34.4	- - - - -	± - - - -
6	- - - - -	4 3 - - - -	26	- - - - -	4 - - - -
7	- - - - -	4 ± - - - -	38.8	- - - - -	- - - - -
8	- - - - -	4 2 - - - -	35.4	- - - - -	4 ± - - - -
9	+ - - - -	4 4 2 - - -	45	- - - - -	4 4 - - - -
10	± - - - -	4 4 - - - -	24	± - - - -	4 4 - - - -
11	- - - - -	4 4 - - - -	37.2	- - - - -	4 3 - - - -
12	4 3 ± - - -	4 4 4 - - -	24	3 2 ± - - -	4 4 4 - - -
13	4 3 - - - -	4 4 4 4 - -	58	4 2 - - - -	4 4 4 4 - -
14	4 4 4 4 - -	4 4 4 4 - -	46	4 4 4 ± - -	4 4 4 4 - -
15	4 4 - - - -	4 4 4 4 - -	51.2	4 3 - - - -	4 4 4 4 - -
16	4 4 4 4 - -	4 4 4 4 - -	57	4 4 4 4 - -	4 4 4 4 - -

for a sufficiently high icterus to block completely complement-fixation in low-titered syphilitic serums to give a false-negative report even with the cardiolipin-lecithin Kolmer antigen, but in such a case the amount of icterus necessary was greater than that required to give a false negative report with the regular Kolmer antigen.

In serums containing 16 cardiolipin-lecithin Kolmer units an icterus index sufficiently high to block complement-fixation completely was not found, but it is believed from this study that with 8 or less cardiolipin-lecithin Kolmer units an icterus index above 44 units could cause a false-negative Kolmer report with the use of the regular Kolmer antigen, whereas the report would be positive in tests using the new cardiolipin-lecithin antigen.

Negative serums with an icterus index as high as 80 units failed to fix complement.

CONCLUSIONS

The cardiolipin-lecithin Kolmer antigen test is more sensitive than the regular Kolmer antigen in testing icteric low-titered syphilitic serums.

Icteric serum, *per se*, is not capable of fixing complement; the active syphilitic reagin is necessary for complement-fixation whether icterus is present or not.

The use of cardiolipin-lecithin antigen in complement-fixation tests on highly icteric serums does not result in false positive reports. On the contrary, the presence of icterus in the serum may completely block fixation of complement in regular Kolmer antigen tests; and it may inhibit somewhat complement-fixation in cardiolipin-lecithin Kolmer antigen tests but not to the point where it would give a false negative report in high-titered serums. A sufficiently high icterus is capable of blocking complement-fixation completely in a serum having a low titer of syphilitic reagin.

PHENOL RED BROTH MEDIUM ENRICHED WITH RABBIT SERUM FOR *NEISSERIA GONORRHOEAE* FERMENTATIONS*

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Attempts to determine fermentation reactions of strains of *Neisseria gonorrhoeae* have been relatively unsuccessful because of lack of a suitable cultural medium. Various animal fluids have been suggested as enrichment for growth of the Gonococcus. Carpenter² states that ascitic fluid agar is the best medium for detection of fermentation reactions. Ascitic fluid, however, must be bile-free with a pH range between 7.2 and 8.0. Some lots of ascitic fluid, even though meeting these specifications, may still be unsatisfactory and, therefore, it is necessary to test each new lot with known organisms. Peizer⁴ recommends a 0.3 per cent semisolid proteose peptone agar medium enriched with 5 per cent of human, guinea pig, or rabbit serum plus 0.5 per cent of the carbohydrates to be tested. We have found that 0.5 per cent carbohydrate does not give consistent results.

The phenol red broth medium with 0.2 per cent agar recommended by Difco³ as a base medium for fermentation reactions of the Gonococcus also has given inconsistent results in our hands. However, this medium enriched with rabbit serum and prepared exactly as specified below has been found to give duplicable results.

PREPARATION OF MEDIUM

Prepare phenol red broth in a semisolid form by adding 0.2 per cent Bacto agar. Sterilize at 121 C. for fifteen minutes. Sterilize 10 per cent aqueous solutions of the test sugars by filtration through a Seitz filter. Add the sugar to the medium to make a concentration of 1 per cent. Distribute the sugar medium aseptically into sterile agglutination tubes in 4 ml. amounts and incubate these tubes for twenty-four hours at 37 C. to test sterility. The tubes may then be stored in the refrigerator until needed. Before use, boil the tubed medium five minutes and cool without excessive agitation to approximately 60 C. To each tube of cooled medium add 0.2 ml. of sterile rabbit serum previously inactivated by heating to 60 C. for ten minutes. Unless the serum is heat-inactivated, results will be inconsistent.

FERMENTATION TESTS

Emulsify the growth of a forty-eight-hour chocolate agar slant culture of *N. gonorrhoeae* in approximately 0.5 ml. of sterile saline with a sterile pipet. Use the same pipet to transfer 2 or 3 drops of the suspension to each tube of carbohydrate medium. Incubate the inoculated tubes at 37 C. Most cultures may be

* Received for publication, November 13, 1947.

expected to be positive in forty-eight hours but, if necessary, incubation should be continued for seven days before reporting them as negative. Check the reaction frequently because acid production may be transient, especially with recently isolated cultures of *N. gonorrhoeae*.

RESULTS

Dextrose, maltose, sucrose, levulose and lactose have been tested by the above method, using 55 different cultures of *N. gonorrhoeae*. Forty-nine strains were freshly isolated and six were taken from a group of dried sulfonamide-resistant strains. In addition one strain each of *N. catarrhalis* and *N. sicca* and three strains of *N. intracellularis* (Groups I, II and II *alpha*) have been used. All these cultures grew luxuriantly on the medium. All *N. gonorrhoeae* cultures fermented dextrose only, in from one to six days; the other *Neisseria* organisms gave typical and consistent fermentation reactions. One culture of the dried sulfonamide-resistant strains took six days to ferment dextrose, the longest time required for any culture to ferment this sugar. Bahn, Ackerman and Carpenter¹ have shown that penicillin-resistant strains of the *Gonococcus* give delayed fermentations of dextrose. It is possible that the sulfonamide-resistant strains may show the same tendency.

Since the completion of the work reported above, an additional 100 strains of *N. gonorrhoeae* have been tested and confirmed by this method.

This medium is recommended for the determination of fermentation reactions of *N. gonorrhoeae* because of the ease of preparation (the basic medium is available in commercial dehydrated form and rabbit serum is readily obtainable in most laboratories) and because, with a few precautions, it gives completely reliable results.

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NEWS AND NOTICES

ANNUAL LETTER TO REGISTERED MEDICAL TECHNOLOGISTS

Dear Registrants:

The following report is submitted in place of the annual letter which, in the past, has been mailed with the renewal forms for registration. It is the hope of the Board of Registry that this report will give a better understanding of the work of the Registry.

Growth in the Number of Medical Technologists

Year by year, the number of registered medical technologists has increased at almost exactly the same rate, but there are indications that this rate will increase within the next year or two. There are now 11,349 registered medical technologists; 8861 of these had renewed their certification as of December 1, 1947.

Examination of Candidates for Registration

During the past year, 1324 candidates were scheduled for examination, 68 failed to appear at the examination, 999 were certified as a result of the examinations and 257 failed. A total of 378 examiners cooperated in giving the two examinations. As in the examination given in the Fall of 1946, the examinations given during the past year were made up, in part, of true and false questions, and in part, of the usual type of written paper. Careful statistical analysis of the results has shown beyond any doubt that the true and false type of examination is as effective, and in some ways, more effective than the written paper. The essay type of paper which has been standard for many years has made it possible for the Registry to know almost exactly the general outcome of any given examination. A comparison with the results of the well established standard examination has shown that the true and false test is a perfectly fair test and in some ways is superior since it covers a wider field of knowledge.

Occasional objections have been raised to the discontinuance of the practical examination. It has been mentioned before, and it is now reaffirmed by the Board, that although the practical examination was an excellent method of evaluating the technical skill of candidates, it was quite ineffective in providing any pertinent information regarding the candidate's ability beyond that gained on the basis of the written paper. The fact that the examinations were conducted in a great number of centers rendered the findings nonuniform, and so the Board believes it inadvisable to re-institute the practical examination. The determination of the students' ability is actually the responsibility of the pathologists who train them and it is believed that a year's observation in this way is likely to reveal the ability of the students. In order to determine what evaluation the teachers of these candidates have placed on them, the Registry requires that all candidates for examination submit satisfactory recommendations from two pathologists who have observed their work.

The Registry as a Qualifying Body

It has been pointed out many times that the Registry of Medical Technologists is a qualifying body paralleling in almost every way the so-called qualifying boards of the medical specialties. It is not a society; it is not an association; it has no possible resemblance to a labor union. The Registry is, in fact, designed for one purpose and one purpose only, *viz.*, the identification, by means of suitable credentials and examinations, for certification as medical technologists, those candidates who have proper qualifications according to the present minimum requirements.

True enough, the Registry has, from time to time, temporarily assumed other functions which were necessary for one reason or another, but which were outside the strict limits of its official responsibility. As time has passed, however, several of these functions gradually have been assumed by the American Society of Medical Technologists, which is the national association of registered medical technologists. This is a professional society performing many functions which cannot be assumed by the Registry, either because they are outside its sphere or because the Registry has neither facilities nor opportunity to carry them on. One of these responsibilities which has been assumed gradually by the American Society of Medical Technologists is the stimulation of interest in post-graduate courses and post-graduate education in general. In the past year the Registry provided funds for carrying out this work, through the Committee on Education of the American Society of Medical Technologists. With the help of allocations from the Educational Fund set up by the Registry, fourteen post-graduate seminars were held, with approximately 2200 medical technologists in attendance from at least 25 states. This work has been so successful that the Registry has set aside funds for this purpose for the ensuing year.

Code of Ethics

During the past year a committee consisting of representatives from the Board of Registry and the American Society of Medical Technologists has made an exhaustive study of the codes of ethics of other organizations analagous to the American Society of Medical Technologists and qualifying bodies such as the Registry. As a result of this study, a more equitable code of ethics has been designed, and this will be published in a subsequent issue of the Technical Bulletin. An effort has been made to define more fully in this code some provisions which were less explicit in the previous code. It is apparent, however, that an exact definition of the limitations of some points in any code of ethics may not fit every circumstance and thus may require individual interpretation.

Questionnaire

Because of repeated demands for a decision on the matter of other standards of certification of laboratory workers, as well as demands for information on various aspects of medical technology, the Board of Registry during the past year submitted to pathologists and registered medical technologists in equal

numbers, a questionnaire which was designed to provide answers for some of these questions. Space does not permit a complete discussion of the questionnaire at this time, but in brief, the answers to it indicate the following:

1. The majority favors the establishment of a so-called "junior" grade of medical laboratory worker.

2. A great majority believes that the present standards of certification of medical technologists are satisfactory, both in regard to pretechnical and technical training. A few think that the standards are too high, and a potent minority believes that they are too low.

3. The large majority is opposed to licensing of laboratory workers by the states.

4. The majority favors the present method of renewal certification by having the form signed by the physician who supervises the work of the medical technologist.

There is no doubt that some will be much opposed to the changes to be made as a result of the answers to the questionnaire. However, since the questionnaire was submitted to such a large group in the field of laboratory medicine, the Board believes that these changes are justified.

Technical Bulletin

The large number of papers submitted for publication made it necessary and desirable to increase the size of the Technical Bulletin by approximately fifty pages for the year. This made it possible to publish much material which otherwise would have been delayed because of lack of space. Furthermore, the format has been changed to conform to that of the American Journal of Clinical Pathology. It has long been apparent that the Technical Bulletin has filled a need for a publication which finds its way to all registered medical technologists.

Certification of Service-Trained Technologists

In the past, the Board of Registry has made an effort to certify all service-trained medical technologists who met the minimum pretechnical and technical training requirements. The Board has thus encouraged and assisted these workers to establish themselves on a professional basis in civilian life. It is gratifying to note that of all groups taking the examination, those who received their training in the armed services had the smallest percentage of failures.

The Registry and the American Society of Medical Technologists

A high degree of cooperation and collaboration has been maintained between the Registry and the American Society of Medical Technologists. During the past few years, the Board has been assisted in its deliberations by an Advisory Committee of registered medical technologists, three of whom are members of the American Society of Medical Technologists, and three of whom are appointed to represent the registrants at large. During the past year this assistance has

been further extended by two representatives of this committee who sat in at meetings of the Board.

The Chairman of the Board had the pleasure of attending the meeting of the American Society of Medical Technologists in Denver in June 1947. The fine registration at this meeting, the excellent program exhibits and the progressive and constructive spirit of the officers and members, bodes well for the future of this Society, as it takes its place among national professional organizations, and as it takes all possible means to maintain the practice of medical technology on a high level in this country.

In conclusion, the Board of Registry and the staff of the Registry office take this opportunity of extending their greetings to all registered medical technologists. It is the desire of the Board to render all possible assistance in conducting the work of the Registry and in maintaining the high standards in laboratories in which medical technology is performed.

With best wishes, I am

Sincerely yours,

LALL G. MONTGOMERY, M.D.
Chairman, Board of Registry

UNKNOWN ADDRESSES OF REGISTERED TECHNOLOGISTS

Following is a list of registered Medical Technologists whose present addresses were unknown in the Registry office as of November, 1947. Because of lack of space the last known full address is not given. Any information regarding the complete present address of any of the following registrants will be gratefully received.

ALABAMA

Blosser, Mrs. Ruth Guggenheim, Gadsden

Brown, Mrs. Chloette O., Birmingham
Filip, Mrs. Eleanor S., Maxwell Field
Handley, Mrs. Edith Kinkead, Birmingham

ARIZONA

Bayless, Mrs. Donalda M., Bisbee
Brown, Mrs. Roberta Carl, Phoenix
Miller, Mrs. Ruth C., Phoenix
Smith, Miss Eileen, Phoenix
Warren, Mrs. Betty Smith, Phoenix

ARKANSAS

Condell, Mrs. Eugenia B., Little Rock
Kersbergen, 1st Lt. Garrett, Hot Springs

Kilbury, Mrs. Laura K. Farabee, Little Rock

Ponder, Miss Joyce Perry, Little Rock

CALIFORNIA

Barsoom, Mrs. Jane Swalwell, Reedley
Bauer, PhM2/c Adelia Catherine, San Diego

Blaga, Miss Cornelia A., San Diego
Buchanan, Miss Dorothy R., Los Angeles

Bueter, Miss Lois Elinor, Los Angeles
Burr, Mrs. Margaret S., San Francisco
Carr, Miss Anita Marie, Pasadena
Clark, Mrs. Elizabeth Ann, San Leandro

Daniel, Miss Alice, Modesto
DesRosier, Miss Geraldine J., San Francisco

Dragseth, Miss Julia C., San Rafael
 Eisen, Mrs. Ruth, San Francisco
 Englund, Miss Janet M., Palm Springs
 Espey, Ensign Harriet Bloom, San Francisco
 Goldberg, Miss Gussie G., San Francisco
 Graham, Mr. Angus Riddle, Los Angeles
 Grant, Mrs. Patricia P., Pomona
 Gross, Mrs. Bernice Bache, San Francisco
 Holm, Miss Ruth V., Inglewood
 Kalina, Miss Mary T., Stanford University
 Lind, Mrs. Helen A., San Jose
 Makins, Miss June M., Stanford University
 Markel, Mrs. Wilma S., Trona
 Martin, Mrs. Laurabel G., Palo Alto
 Maxwell, Mrs. Ruth L., Plaza Del Rey
 McDonald, Miss Marjorie, San Francisco
 Miller, Lt. (jg) Hazel H., Santa Cruz
 Miller, Mrs. Mary Ward, Long Beach
 Mitchell, Mrs. Jane Spalsbury, Long Beach
 Moorhead, Mr. Zenith D., San Francisco
 Mullan, Miss Imogene L., Los Angeles
 Pess, Mrs. Anne R., Long Beach
 Phillips, Mrs. Ann M., Richmond
 Pizio, Mrs. Irma C., National City
 Prael, Mr. William J., Berkeley
 Russell, Mrs. Margaret Brewer, Modesto
 Schlichting, Lt. (jg) Virginia May, San Francisco
 Schmidt, Miss Eileen, San Francisco
 Shugarman, Miss Anita Rae, Venice
 Stricklin, Mrs. June Benham, Los Angeles
 Ureen, Miss Helen Janet, San Francisco

Van Sicklin, Mrs. Dorothy W., San Diego
 Weisskopf, Miss Julia B., Palm Springs
 White, Miss Virginia Lee, Los Angeles
 Williams, Miss Vera I., Coachella
 Young, Ensign John L., San Diego

COLORADO

Braxmeier, Miss Ludmilla, Denver
 Dieringer, Miss Kathleen Ellen, Denver
 Kokesch, Pvt. Ruth Frances, Denver
 Lewis, Miss Charlotte Lucy, Colorado Springs
 Piper, Miss Amanda L., Fort Collins
 Schiffour, Mrs. Dorothy M., Denver
 Sexton, Mrs. Lois Allen, Denver
 Wallace, Miss Marjorie J., Gunnison

CONNECTICUT

Kemp, Mrs. Ruth E., New Haven
 Schoendorf, Miss Jean Mary, Waterbury
 Winters, Mrs. Elizabeth M., Norwich

DISTRICT OF COLUMBIA

Adams, Mrs. Ruby A., Washington
 Ralya, Mrs. Joan D., Washington
 Sidwell, Mrs. Catherine Aye, Washington
 Stratton, Mrs. Elberta L., Washington
 Trippe, Mr. Alton Dorsey, Washington

DELAWARE

Buffington, Miss Estella, Wilmington
 Hirsch, Mrs. Sylvia G., Lewis

FLORIDA

Boyd, Mrs. Marlys Harris, St. Petersburg
 De Court, Miss Alma E., Lake Worth
 Ellsworth, Miss Marian Allene, Miami
 Hickman, Miss Margaret Elizabeth Miami

Hoagland, Mrs. Harriet A., Jacksonville

Meltzer, Mrs. Ruth Rae, Miami

Patterson, Miss Edythe Larue, Coral Gables

Shafer, Lt. Melba V., Tallahassee

Tabak, Mrs. Rhea Mallinger, Miami

GEORGIA

Blalock, Mrs. Jean W., Atlanta

Hillis, Miss M. Senie, Atlanta

McAdams, Lt. Mary Virginia M., Fort Benning

Price, Mr. Clifford D., Atlanta

Welch, Miss Margaret, Atlanta

IDAHO

Weeks, Mrs. Helen Fouke, Twin Falls

ILLINOIS

Babbin-Zverow, Mrs. Mildred, Chicago

Bolender, Miss Marjorie, Rockford

Chavin, Miss Eleanor, Chicago

Cleary, Miss Margaret, Oak Park

Clune, Miss Margaret Ann, Quincy

Dietz, Mrs. Ruth Hart, Chicago

Gant, Miss Virginia G., Aurora

Garlock, Mr. Fred C., Rantoul

Jenkins, Mrs. Gwendolyn McNiece, Chicago

Knisely, Miss Mary Jane, Chicago

Kubon, Miss Shirley Etta, Chicago

Lance, Mrs. Ruth Hinkle, Calumet City

McLaughlin, Mrs. Laura Coe, Springfield

Pearlstein, S/Sgt. Mildred, Hines

Pohlen, Miss Marion D., Chicago

Remain, Sister Mary Ignatius, Chicago

Rouse, Miss Opal Mae, Galesburg

(Sappok), Sister Benita, Springfield

Sasaki, Miss Myrtle Hazue, Chicago

Shirley, Mrs. Pauline B., Indianola

Sieren, Sister M. Leonilla, Chicago

Smith, Miss Ellen M., Chicago

Sunderland, Miss Helen E., Chicago

Wood, Mr. Glenn M., Chicago

INDIANA

Bartholome, Mrs. Lynn Dauderman, Terre Haute

Hammes, Mrs. Anne G., Anderson

Lynn, Mrs. Lois P., Gary

Sargeant, Miss Barbara Ann, West Lafayette

Swaim, Mrs. Malala H., Lafayette

IOWA

Amende, Miss Jeanette, Iowa City

Farley, Miss Barbara, Laurens

Francis, Miss Marion C., Davenport

Gilmor, Mrs. Margery M., Ames

Johnson, Mrs. Eleanor S., Iowa City

Varney, Mrs. Janice N., Iowa City

KANSAS

Carter, Miss Marian Berniece, Manhattan

Eaton, Mrs. Rebecca G., Herington

Harmon, Mrs. Nila Gentry, Lawrence

Spearman, Miss Marion Minerva, Kansas City

Strong, Mrs. Martha Y., Leavenworth

KENTUCKY

Brydon, Miss Martha Lou, Lexington

Corbett, Miss Charlotte, Lebanon

Crowe, Miss Madalyn Marie, Louisville

Heim, Mrs. Mary Volk, Louisville

Knittel, Mrs. Dora Cooper, Louisville

Luther, Miss Dorothy, Hopkinsville

McCarthy, Miss Virginia Marion, Lexington

McClung, Mrs. Genelle B., Louisville

Moore, Mrs. Lucy Hume, Louisville

O'Daniel, Mrs. Clara Scott, Louisville

Ries, Mrs. Mary M., Lexington
 Rowe, Miss Evelyn, Louisville
 Royalty, Mrs. Winifred T., Elizabeth-
 town
 Santiago, Miss Nellie, Louisville
 Shreve, Miss Tommie Mary, Louisville

LOUISIANA

Boucher, Mrs. Jane R., New Orleans
 Caulfield, Miss Edna, Shreveport
 Charbonnet, Mrs. Rose Mary H., New
 Orleans
 Farmer, Mrs. Elizabeth, Lafayette
 Hunter, Miss Annie Mary, New Or-
 leans
 Hutton, Mrs. Nola Granger, New
 Orleans
 Ingram, Miss Leta Byrd, DeRidder
 Moody, Mrs. Eileen M., Baton Rouge
 Shea, Mrs. Eula Jones, Shreveport

MARYLAND

Friedman, Miss Loraine, Baltimore
 Huth, Miss Jayne Lucille, Baltimore
 Kelly, Mrs. Juanita L., Cumberland
 Parrett, Mr. Frederick W., Annapolis
 Polek, Mrs. Virginia Hardin, Baltimore
 Price, Miss Mildred E., Baltimore
 Sheriff, Miss Janice, Baltimore

MASSACHUSETTS

Biron, Mrs. Marie S., Ayer
 Marshall, Miss Virginia, Boston
 Schuster, Mrs. Frances S., Holyoke
 Sweeney, Miss Loretta N., Camp
 Edwards

MICHIGAN

Bassani, Miss Eugenia L., Ann Arbor
 Cannon, Miss Mary R., Grosse Pointe
 Farley, Miss Elizabeth A., Detroit
 Feller, Miss Mary E., Bay City
 Hochstetler, Miss Sara Kathryn,
 Benton Harbor
 Hutchins, Mr. Ralph C., Bay City

Pearson, Miss Frances K., East Lan-
 sing
 Posey, Mrs. Wanda F., Detroit
 Schaeffer, Miss Alice Bell, Detroit
 Titchenal, Mrs. Ethel Stead, Detroit
 Trebilcock, Miss Virginia Ann, Detroit
 Vance, Miss Eugenia S., Detroit

MINNESOTA

Blamey, Mrs. Dorothy O., Minneapolis
 Breneman, Mrs. Janet J., Minneapolis
 Frantz, Miss Marthella J., Minneapolis
 Gabriel, Miss Virginia Lee, Minne-
 apolis
 Goto, S/Sgt. Kenneth Yoneji, Fort
 Snelling
 Gouze, Miss Mary Louise, Minneapolis
 Harvey, Mrs. Dolores Jean, Minne-
 apolis
 Hoilund, Miss Lucille J., Minneapolis
 Howe, Mrs. Marian P., St. Paul
 Johnson, Miss Phyllis M., Minneapolis
 Meyers, Mrs. Zona Brandt, St. Paul
 Radway, Mrs. Elizabeth, Minneapolis
 Scholljegerdes, Miss Virginia R., Min-
 neapolis
 Syverson, Miss Doris Meld., Minne-
 apolis
 Walburg, Miss Betty Lou, St. Paul

MISSISSIPPI

Erickson, Mrs. Phyllis B., Biloxi

MISSOURI

Altemeier, Mrs. Alice Robinson, St.
 Louis
 Haskins, Mrs. Thelma Brewer, St.
 Louis
 Heins, Jr., Mrs. Lucia F., St. Louis
 Lanbert, T/4 Margaret F., Springfield
 Moorman, Miss Pearl L., Kansas City
 Nance, Mrs. Luciel, Kansas City
 Rounds, Mrs. Martha M., St. Louis
 Svoboda, Mrs. Betty W., Kansas City

MONTANA

Davenport, Sister Bernadette D.,
Great Falls
Maffei, Miss Marie C., Billings
Sheehy, Mrs. Rita Ann Schiltz, Helena

NEBRASKA

Anstine, Mrs. Jean Foutch, Lincoln
Brown, Mrs. M. Elise W., Omaha
Murphy, Miss Ada, Lincoln
Tabaka, Miss Frances C., Omaha

NEW JERSEY

Bigelow, Mrs. Dorothea W., Bound
Brook
McCutchen, Mrs. Nell K., New Bruns-
wick
Spencer, Mrs. Catherine W., Trenton

NEW MEXICO

Beattie, Mrs. Mary A., Las Vegas
Collins, Mrs. Rita C., Albuquerque
Miller, Miss Evelyn M., Santa Fe
Norris, Mrs. Madeleine, Santa Fe
Wiltse, Miss Fern L., Santa Fe

NEW YORK

Bauman, Mrs. Evelyn Jane, White
Plains
Deane, Lt. Hazel F., New York City
Durlham, Sgt. Lowell J., New York
City
Farber, Mrs. Billie F., Rochester
Gold, Mr. E. Allen, New York
Haynes, Miss Anne Malin, New York
City
Jenkins, Sgt. Alta G., Brooklyn
Lowenstein, Mr. Walter Bert, Brook-
lyn
Nathan, Mrs. Ruth Wohl, Buffalo
Paulissen, Mrs. Helen, Willowbrook,
Staten Island
Robinson, Mrs. Mary A., New York
Shaw, Capt. Bernard G., New York

Stankewick, 1st Lt. Walter R., New
York City
Sylvester, C., Pharm. Donald Cyril,
Sampson

NORTH CAROLINA

Baldwin, Mrs. Nancy Minus, Ashe-
ville
Cook, Mrs. Edith Benson, Fayette-
ville
Ferguson, Mrs. Lucy Ayseue, Raleigh
Grimes, Mrs. Doris C., Durham
Hart, Mrs. Ethel Campbell, Winston-
Salem
Marvin, Miss Sara Kathleen, Charlotte
Riggall, Mrs. Frances Adkins, Durham

NORTH DAKOTA

Bahr, Miss Ellen M., Bismarck

OHIO

Ackard, Mrs. Patricia S., Dayton
Caplan, Mrs. Eleanor K., Cleveland
Clear, Mrs. Mary Malloy, Cincinnati
Cooper, Mrs. Isabelle U., Columbus
Feo, Mrs. Mildred Edith, Cleveland
Gwinner, Miss Georgia E., Columbus
Harriger, Miss Donna Dalton, East
Liverpool
Lloyd, Mrs. Dorothy K., Cleveland
Olms, Mrs. Helen W., Shaker Heights,
Cleveland
Richardson, Miss Lucille, Toledo
Rimer, Mrs. Valeria, Moser, East
Cleveland
(Rothermel), Sister M. Eulalia, Canton
Siemon, Mrs. Carmen A., Cincinnati
Trogler, Miss Anna Ruth, Columbus

OKLAHOMA

Dandridge, Mrs. Jane R., Oklahoma
City
Henry, Mrs. Mildred Wall, Oklahoma
City

OREGON

Betz, Mrs. Beth Mitchell, Portland
Brownfield, Mrs. Marjorie M., Ft.
Stevens

Kunsch, Miss Mary Dorothy, Portland
(Lander), Sister Louis Eugene, Med-
ford

Packard, Miss Dorothy, Astoria

PENNSYLVANIA

Adams, Mrs. Rebecca Finnie, Hunt-
ingdon Valley

Aye, Mrs. Mary Otte, Brackenridge

Beach, Mrs. Jane Heading, Pittsburg

Brown, Miss Marjorie Lillian, Bryn
Mawr

Christian, Mrs. Constance K.,
Franklin

de Jesus-Mattos, Mrs. Arcely P.,
Philadelphia

(Dorochowicz), Sister Mary Carmella,
Pittsburg

Lefever, Mrs. Faye Graham, Harris-
burg

Randle, Miss Jane Pierce, Danville

Rich, Mrs. Dorothy Koch, Phila-
delphia

Shoemaker, Miss Justina Ann,
Pittsburgh

Steward, Miss Lucille Ruth, Hazelton

Woerner, Mrs. Elsie H., Lenni Mills-
Delaware Co.

Wright, Mrs. Ruth Sanford, Warren

RHODE ISLAND

Clegg, Lt. Doris L., Newport

SOUTH CAROLINA

Bohland, T/5 Marie, Fort Moultrie

Hancock, Mrs. Dorothy K., Sumter

Marshall, Miss Fannie Alice,
Charleston

TENNESSEE

Calliham, Mrs. Sarah G., Memphis
Smith, Miss Elizabeth J., Knoxville

Swartz, Mrs. Evelyn M., Memphis
Weis, Mrs. Emmette Jo, Memphis
Wilson, Miss Elizabeth D., Knoxville

TEXAS

Allen, Miss Elizabeth R., El Paso

Beeman, Miss Joyce, Dallas

Brittnacher, Mrs. Mary Butler,
Houston

Dickie, Miss Sarah Jane, Houston

Dunnam, Mrs. Betty W., Houston

Funk, Mrs. Elizabeth S., Port Arthur

Hyams, Mrs. Florence D., Dallas

Jack, Miss Elizabeth Ann, Brownsville

Johnson, Mrs. Ann Adele Crane,
Dallas

Kennedy, Mrs. Virginia S., Galveston

Lang, Mrs. Neenah J., San Antonio

Langston, Mrs. Nellie Belden, Gal-
veston

Marshall, Mrs. Winston Day,
Weatherford

Matson, Miss R. L., Dallas

Murphy, Miss Mary Jean, Galveston

Nabb, Mrs. Sadie Polk, San Antonio

Neill, Mrs. Martha Jane, Houston

Orr, Lt. Marjorie K., Houston

Pike, Mrs. Lena Hess, Houston

Pinckard, Miss Betty Belle, Houston

Podolsky, Mr. Jose Lijovetsky, Gal-
veston

Randolph, Miss Olivia Lee, Houston

Taylor, Mrs. Bettie Lee, Dallas

(Valdez), Sister M. Inez, Lubbock

Walters, Mrs. Marion L., Dallas

UTAH

Carlson, Mrs. Ellen Braxtan, Brigham
City

Faulds, Miss Arlene Marjorie, Salt
Lake City

Seavers, Mrs. Margaret M., Salt Lake
City

Spickard, Mrs. Ida Merrill, Salt Lake
City

VERMONT

Walter, Miss Clara L., Burlington

VIRGINIA

Evans, Miss Eloise J., Roanoke
Fisher, Miss Teresa Corinne, Richmond

Funk, Mrs. Frances Gill, Roanoke
Godwin, Mrs. Mildred C., Hampton
Hester, Mrs. Marie S., Norfolk
Latson, Mrs. Grace H., Arlington
McCutcheon, Mrs. Rebecca M., Norfolk

Salvador, PhM2/c Isabel L., Norfolk
Whittle, Mrs. Margaret Stuart, Charlottesville
Williams, CPhM Vernon E., Portsmouth

WASHINGTON

Adatto, Miss Jennie Q., Seattle
Anderson, Mrs. Helen R., Walla Walla
Blauvelt, Miss Barbara, Richland
Curry, Mrs. Mary Creeson, Spokane
Dedrickson, Miss Mildred A., Seattle
Foster, Mrs. Frances C., Seattle
Haas, Miss Mary Lou, Bremerton
Helleckson, Miss Mariann A., Seattle
McDaniel, Mrs. Dorothy Lang, Seattle
Potter, Mrs. Mary Reynolds, Walla Walla
Shoemaker, Ensign William C., Seattle
Woolson, Mrs. Elizabeth Jane B., Spokane

WISCONSIN

Bielfeldt, Miss Mary Ellen, Milwaukee
Fechter, Miss Mary Jane, Milwaukee

Holland, Miss Helen L., Oakfield
John, Mrs. Grace K., Milwaukee
Kurta, Mrs. Virginia Malone, Milwaukee

Laabs, Mrs. Gail Frostad, Madison
Linkert, Sister Ursitia M., Sheboygan
Miller, Mrs. Evelyn Clarke, Madison
Nelson, Mrs. Jeannette, Stevens Point
Pauly, Mrs. Ardele M. Boland, LaCrosse
Walker, Miss Irma Jane, Sheboygan
Washburn, Mrs. Dorothy Kohlhepp, Milwaukee

CANAL ZONE

Sears, Miss Mary Elaine, Ancon

HAWAII

Cross, Mrs. Helen Douglas, Schofield, Oahu
Foy, Mrs. Pauline K. Nagaue, Honolulu
Lerner, Mrs. Rebecca W., Honolulu

FOREIGN

Gramsch, Ensign W. H., Samar, P.I.

CANADA

Friesen, Mrs. Eira A. Charles, Winnipeg, Manitoba
Harvey, Miss Margaret W., Winnipeg, Manitoba
Lynch, Miss Euphemia, Windsor, Ontario
Thomas, Miss A. Ruth, Belleville, Ontario
Cayer, Miss Marie Eva, Quebec
Richard, Sister Mary Albert, Lachine, P. Q. Montreal

DECEASED REGISTRANTS

Notices of the death of the registrants listed below were received in the Registry Office during 1947. In this list, the name of the registrant is followed by her (his) registration number, year of registration and last known address.

Miss Dorothy Patterson Barrus (1702), registered 1933, 4111 Pine Street, Philadelphia 4, Pennsylvania.

- Sister M. Isidore Beete (549), registered 1931, Providence Hospital, Sandusky, Ohio.
- Mr. Eldred Marvin Berryman (3877), registered 1937, 801 Power & Light Building, St. Petersburg 5, Florida.
- Miss Beatrice Margaret Bloomquist (9787), registered 1944, 301 Grace Street, Apartment #1, Houston 3, Texas.
- Miss Cameron E. Cameron (5593), registered 1939, 220 17th Street, Pacific Grove, California.
- Sister M. Cherubine Castrop (443), registered 1929, St. Mary's Ringling Hospital, Baraboo, Wisconsin.
- Mr. Howard Ernest Holtzman (2613), registered 1935, 4617 Pinewood Road, Jacksonville, Florida.
- Miss Bessie Horowitz (3638), registered 1937, 419 North Soto Street, Los Angeles 33, California.
- Mrs. Gertrude Ebers Hughes (3007), registered 1936, 721 West 14th Street, Grand Island, Nebraska.
- Mr. Robert Canfield Jenkins (1049), registered 1932, 55 East Washington Street, Suite 1539, Chicago 2, Illinois.
- Mrs. Gertrude Tangen Kerns (1249), registered 1933, 421 Michigan Street, Toledo 2, Ohio.
- Sister Marie Anne L'Ecuier (10020), registered 1944, Holy Ghost Hospital, Laboratory, 1575 Cambridge Street, Cambridge, Massachusetts.
- Mrs. Elsie Meredith Maxon (10483), registered 1944, 41 Evergreen Street, Rochester 5, New York.
- Mrs. Mary Jane Roh Moline (11419), registered 1945, 49 Fir Hill, Akron 4, Ohio.
- Mr. Chester Alan Peyton (4933), registered 1938, San Antonio, Texas.
- Miss Ruth Sydney Peterson (6200), registered 1940, St. Elizabeth's Hospital, Madison Avenue and 21st Street, Granite City, Illinois.
- Miss Anna Marie Reilly (3418), registered 1936, 130 Osborne Street, Wissahickon, Philadelphia 28, Pennsylvania.
- Miss Charlotte Virginia Stevens (12495), registered 1946, 907 Third Street, Bay City, Michigan.
- Mr. Henry Louis Thompson (5856), registered 1939, 124 Bond Street, Astoria, Oregon.
- Miss Margaret Anna Wiedemann (1276), registered 1933, 344 Ogden Avenue, Jersey City, New Jersey.
- Miss Doris Lee Wood (10251), registered 1944, Davis Hospital, Statesville, North Carolina.
- Mrs. Mary Thomas Wright (1192), registered 1933, 540 Thorn Street, San Diego 1, California.

COURSES IN LABORATORY DIAGNOSIS OF PARASITIC DISEASES

Six-week refresher courses for laboratory personnel in the Laboratory Diagnosis of Parasitic Diseases will be offered by the Laboratory Division of the Communicable Disease Center of the United States Public Health Service, from July 12 to August 30, and from October 11 to November 19. There is no tuition or laboratory fee for these courses, but travel and living expenses must be paid for by the individual or his employer. Laboratory directors and senior staff members wishing to attend any of the six-week courses may do so. However, it is also proposed to schedule one or two short courses for them in the Laboratory Diagnosis of Parasitic Diseases. Definite dates for these two-week classes have not been set. Anyone interested in these courses may obtain further information by writing to Dr. R. F. Reider, Assistant Chief, Laboratory Division, United States Public Health Service, 291 Peachtree Street, Atlanta, Georgia.

RECOMMENDATIONS OF REVIEW BOARD ON NOMENCLATURE OF THE ANTI-Rh TYPING SERUMS

At the request of the Surgeon General of the United States Public Health Service, a Review Board consisting of Dr. Laurence H. Snyder, chairman, Dr. William B. Castle and Dr. Maxwell M. Wintrobe, convened in Washington, October 20 and 21, 1947. Formal evidence bearing on the question of the nomenclature of the Anti-Rh typing serums was presented by eight of the outstanding producers and users of such serums in the United States. The discussions were mainly concerned with the merits of two systems of nomenclature. One of the systems is based on the series of allelic genes proposed by Wiener; the other system is based on the series of three closely linked pairs of genes proposed by Race and Fisher of England. A comparison of the individual antisera of these two systems would be as follows:

<i>Wiener</i>	<i>Fisher-Race</i>
Anti-rh'	Anti-C
Anti-Rh ₀	Anti-D
Anti-rh''	Anti-E
Anti-hr'	Anti-e
Anti-Hr ₀	Anti-d
Anti-hr''	Anti-e

The summary of the points in favor of, and against, each system of nomenclature was presented by the Review Board as follows:

IN FAVOR OF WIENER'S NOMENCLATURE

1. It has priority, being proposed by one of the discoverers.
2. It is in use by many clinicians and research workers, and is understood by them.
3. It is used by nearly all the writers on the subject in the Western Hemisphere.
4. No conclusive evidence has been presented against the theory on which it is based.

AGAINST WIENER'S NOMENCLATURE

1. It does not always specify the antigens present in the type name; and it almost never does so in the genotype name.
2. It has changed rapidly from year to year.
3. It is losing followers among the producers and users of serums.
4. It involves complications, both typographical and genetic, of subscripts, superscripts, numbers, primes, and other symbols.
5. It involves the somewhat doubtful assumption of multiple antigens produced by single genes.

IN FAVOR OF THE FISHER-RACE NOMENCLATURE

1. It always specifies in both type name and genotype name the individual antigens present.
2. It is in wide usage in England, is gaining in usage in the Western Hemisphere, and hence may become the international standard.
3. It is simple and direct, both typographically and genetically.
4. It conforms to a one-to-one correspondence between gene and antigen.

AGAINST THE FISHER-RACE NOMENCLATURE

1. It lacks priority.
2. It is based on a genetic hypothesis which is purely theoretical and for which no clear proof exists: a hypothesis no more tenable on genetic grounds than Wiener's hypothesis.

CONCLUSIONS OF THE BOARD OF REVIEW

The Board has carefully considered the above historical development of the two systems of nomenclature, and has weighed the arguments in favor of and opposed to each system. The Board is forced to the conclusion that for the present a compromise must be made, and both systems must be used on the container labels and package labels. Because the Wiener system has priority and is understood by everyone in the United States concerned with the production and use of anti-Rh serums, the Board recommends that the Wiener terminology appear first on the label, followed by the Fisher-Race terminology in parentheses. Typical labels would then appear somewhat as follows:

Anti-Rh Typing Serum (human)
Anti-Rh₀ (Anti-D)
Slide test
Lot No.
Manufacturer

Anti-Rh Typing Serum (human)
Anti-Rh₀' (Anti-C + D)
.....tube test
Lot No.
Manufacturer

Anti-Hr Typing Serum (human)
Anti-hr' (Anti-c)
Capillary test
Lot No.
Manufacturer

Anti-Rh Typing Serum (human)
Anti-rh' (Anti-C)
Slide test
Lot No.
Manufacturer

The package label and literature might contain, in addition to the above, the description of the test by means of which the serum is to be used.

CLINICAL, FUNCTIONAL AND HISTOLOGIC RESPONSES OF FATTY METAMORPHOSIS OF HUMAN LIVER TO LIPOTROPIC THERAPY*

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Lipotropic therapy has been a major advance in the treatment of liver cirrhosis. Normally, an adequate supply of lipotropic substances (methionine, choline and others) permits phosphorylation of neutral fats to phospholipids, in which form fats are more easily transported from the liver to the various tissues of the body. Inadequate supply or utilization of lipotropic substances, due either to faulty nutrition, hepatic injury or endocrine disturbance, may result in fatty infiltration and eventual cirrhosis of the liver. Most of the experimental work, including that on the relation of lipotropic agents to nutritional cirrhosis, has been performed on animals.^{7, 8, 10, 13} In man, the studies on the effect of lipotropic substances have centered chiefly on the therapeutic results in liver cirrhosis.^{11, 15, 17} There have been few studies dealing with the histologic changes occurring in the livers of human patients following lipotropic therapy.^{2, 6} The majority of investigators reported favorable response, especially in those subjects having large livers. The recently expanded use of the liver biopsy permitted a histologic evaluation of lipotropic therapy in human fatty livers. The present study concerns itself with a comparison of the clinical, functional and histologic responses of patients with fatty livers to lipotropic therapy and the problem as to whether disappearance of fat is associated with histologic, functional and clinical improvement in patients with fatty livers with and without cirrhosis.

METHODS AND MATERIALS

The 15 patients selected for this study had enlarged livers with moderate to severe fatty infiltration as proved by initial needle biopsy. The patients were divided into two groups. Group I consisted of 5 persons without cirrhosis (Table 1), the severe fatty changes in the liver being presumably due to disturbed nutrition (4 chronic alcoholics and 1 pellagra patient). They were essentially asymptomatic, showing no signs of liver disease. Group II consisted of 10 patients with fatty cirrhosis. Most of these presented some of the usual

* Supported by a grant from the Jerome D. Solomon Memorial Research Foundation. Received for publication, November 8, 1947.

signs and symptoms of cirrhosis such as ascites, jaundice, splenomegaly, spider nevi and gastro-intestinal disturbance as well as the laboratory findings of impaired liver function. They received either a high protein diet plus considerable vitamin B complex, or a basic diet plus cystine and choline, or the basic diet plus methionine (Table 1). Although choline plus cystine and methionine may have effects other than lipotropic, in this study it is their lipotropic properties that are being considered.

Before institution of therapy, an initial liver biopsy was taken (by Dr. Donald D. Kozoll) and a series of function tests was performed including cephalin-cholesterol flocculation,³ thymol turbidity¹⁴ and cholesterol esters.⁴ The function tests in all cases and biopsies (Table 1) in 10 of the patients were repeated at regular intervals. The biopsy material was fixed in Zenker-Formalin or

TABLE 1

TYPES OF THERAPY (DAILY AMOUNTS)	NUMBER OF CASES	
	Group I—Nutritional Fatty Liver	Group II—Fatty Cirrhosis
1. High protein-high vitamin B complex; CHO 450 Gm.; protein 200 Gm.; fat 80 Gm.; thiamine 50 mg.; vitamin C 500 mg.; nicotinic acid 300 mg.; Brewer's yeast 45 Gm.....	1	2
2. Choline Gm. 3; cystine Gm. 3; CHO 450 Gm.; protein 80 Gm.; fat 50 Gm.; thiamine 25 mg.; vitamin C 100 mg.; nicotinic acid 100 mg.; Brewer's yeast 12 Gm.....	2	6
3. Methionine Gm. 5; CHO 450 Gm.; protein 80 Gm.; fat 50 Gm.; thiamine 25 mg.; vitamin C 100 mg.; Brewer's yeast 12 Gm.....	2	2
Total number cases.....	5	10
Number of cases with repeat biopsies.....	2	7
Total number biopsies.....	9	27

Carnoy's solution, and histologic sections were stained with hematoxylin eosin, Mallory's aniline blue stain and Gomori's reticulum fiber stain.

This method of approach has advantages in that control periods can be shorter than in clinical investigations. Shorter periods of observation are desirable in view of possible spontaneous remissions which may occur with longer control periods. Even in the shorter periods used in this study, however, the possibility of spontaneous improvement may raise difficulties in evaluating the role of therapy. Another possible source of error or limitation in such a study is the question as to whether the specimen obtained by biopsy is representative of the entire liver. However, if, in a series of observations, some of which were based upon repeated biopsies of the same patient, the same general trend is revealed, the results may be considered reliable.

TABLE 2
CLINICAL, FUNCTIONAL AND MORPHOLOGIC CHANGES BEFORE AND AFTER
LIPOTROPIC THERAPY

	NUTRITIONAL FATTY LIVER		FATTY CIRRHOSIS	
	Before	After	Before	After
Subjective symptoms	±	0	++	±
Objective signs	0	0	++	±
Impairment of functional test	±	±	++	±
Fatty changes	+++	0	+++	0
Necrosis	0	0	+	0
Fibrosis	0	0	+	++
Regeneration	±	+	±	++

TABLE 3
LIVER FUNCTION TESTS BEFORE AND AFTER LIPOTROPIC THERAPY

	FATTY CIRRHOSIS				NUTRITIONAL FATTY LIVER			
	Before		After		Before		After	
	N	Ab	N	Ab	N	Ab	N	Ab
Total protein.....	6	4	7	3	3	1	3	
A/G ratio.....	2	8	6	4	5		3	
Alkaline phosphatase.....	0	10	5	5	3	2	1	2
Total cholesterol.....	10	0	2	8	2	3	2	1
Cholesterol esters.....	6	4	5	5	3	2	2	1
Thymol turbidity.....		6	9	1	3	2	1	2
Serum bilirubin.....	1	9	5	5	5		3	
Bromsulphthalein.....		7	5	4	4	1	3	
Prothrombin time.....	7	3	8	2	5		3	
Cephalin-cholesterol flocculation...	2	8	6	4	5		3	
Urine urobilinogen.....	3	7	7	3	4		3	
Serum urobilinogen.....	7	3	6	4	4		3	

N, normal; Ab, abnormal.

RESULTS

Morphologic Response

Prior to institution of therapy all livers histologically showed moderate to severe fatty changes. Earliest histologic response became evident after two weeks of therapy, and in four weeks practically all livers showed disappearance

or marked diminution of fat. With the disappearance of the fatty globules from the parenchymal cells, increased glycogen storage became evident. The irregularity in size and staining qualities of cytoplasm and nuclei disappeared or became less marked. Regeneration of injured hepatic cells also took place (Fig. 1A, B). Of the 7 cirrhotic patients on whom biopsies were repeated, only 1 showed a decrease in fibrosis and periportal inflammatory activity after treatment. One patient showed no significant change; 5 patients showed no halt in the progression of the mesenchymal reaction (Figs. 2A, 3A). Mallory's connective tissue stain revealed an increase and thickening of collagenous fibers extending from the periportal fields into the nearby surrounding parenchyma. Gomori's reticulum fiber stain showed a change in the reticulum pattern in the vicinity of the portal triads. The individual fibers were more closely spaced, much coarser and showed evidence of sprouting (Fig. 2B, 3B). This indicated new formation of fibers and not mere collapse after reduction of the size of the liver cells due to the disappearance of fat. This increase in connective tissue was associated with a more marked periportal infiltration, consisting chiefly of mononuclear phagocytes, lymphocytes and occasional polymorphonuclear leukocytes.

Clinical Response

No significant difference in the favorable clinical and morphologic responses brought on by the different lipotropic agents was noted in this series. After three weeks of treatment, the originally asymptomatic nutritional fatty group remained well clinically. In the cirrhotic group all but 2 patients claimed to have subjective improvement as evidenced by increased appetite, feeling of well-being and vigor. Objective improvement, as measured by weight change, reduction in ascites, disappearance of jaundice and an improved blood picture, was seen in 12 of the 15 patients.

Functional Response

Considering the function tests as a whole without regard to individual types, the group of nutritional fatty livers showed little functional impairment prior to treatment. Only 16 per cent of all tests (68) performed were found in abnormal ranges (Table 3). The greatest abnormalities occurred in the serum alkaline phosphatase, total cholesterol and cholesterol esters and thymol turbidity. After treatment, there was general improvement in all tests (142) except for the thymol turbidity.

In the cirrhotic group, 67 per cent of all function tests (131) performed were abnormal before treatment, the greatest abnormalities occurring in the cephalin-cholesterol flocculation, thymol turbidity, serum alkaline phosphatase, albumin-globulin ratio, serum bilirubin and urinary urobilinogen. After treatment, only 41 per cent of all tests performed (133) were abnormal; all tests showed approximately equal improvement except for the thymol turbidity, which showed no change.

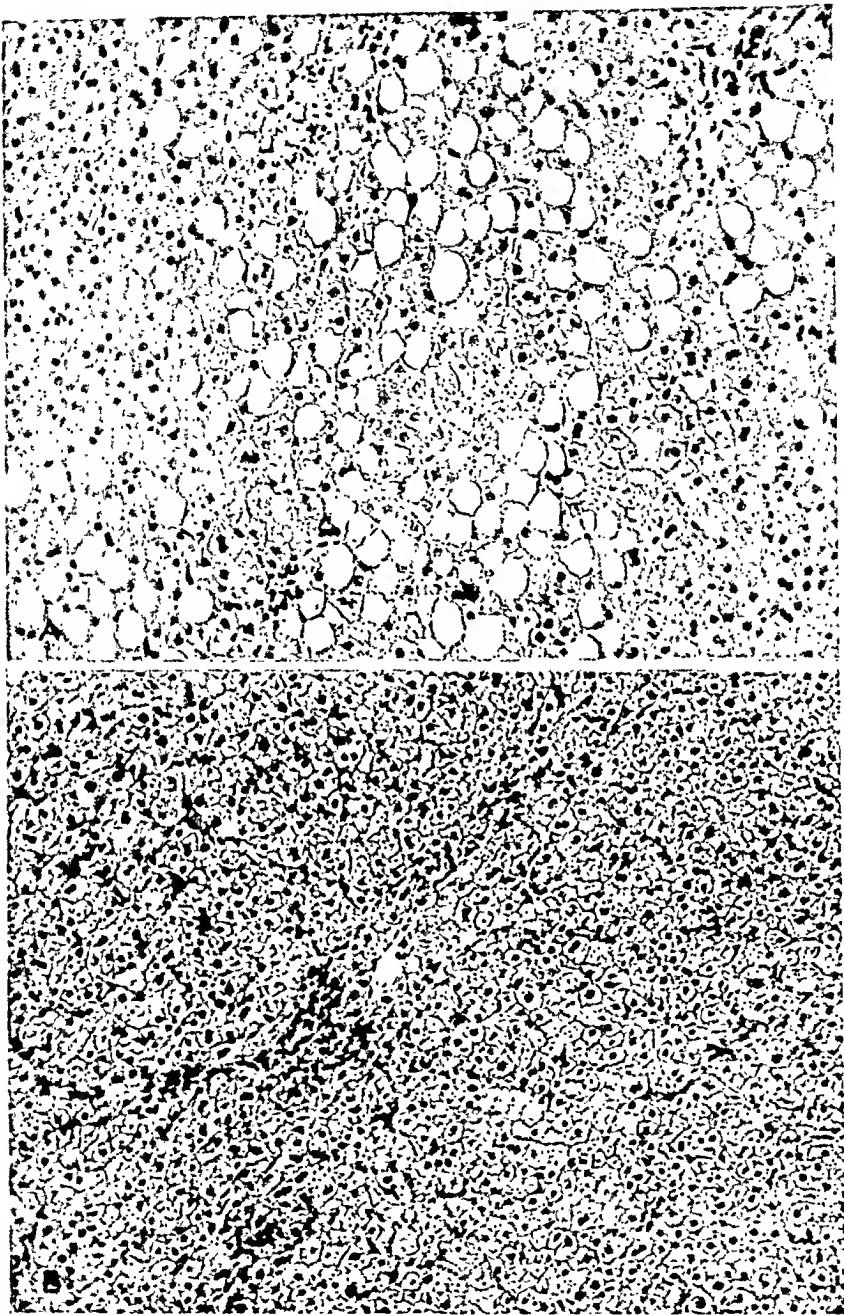


FIG. 1. Liver biopsy specimen of an alcoholic patient who showed evidence of nutritional deficiency. Hematoxylin eosin stain. (A) Before treatment: the arrangement of the liver cell cords is preserved. The cytoplasm of many liver cells is replaced by small and large fat droplets irregularly scattered throughout the cells. (B) Four weeks after treatment with cystine plus choline: the fat droplets have completely disappeared from the cells. The cytoplasm appears vacuolated due to the presence of glycogen.

A striking finding was the consistent elevation of total cholesterol in both groups of patients after lipotropic therapy. The average increase in the nutritional deficiency group was 51 mg. per 100 ml. and in the cirrhotic group, 18 mg. per 100 ml. The cholesterol ester fraction revealed no significant change.

COMMENT

This study presents additional support for the known efficacy of lipotropic therapy in the removal of fat from human fatty cirrhotic and noncirrhotic livers. This was found associated with clinical and functional improvement. No evidence, however, was found that with the removal of fat, the cirrhotic process is arrested.

The clinical and functional improvement of the patients was mirrored in the appearance of the liver parenchyma which showed disappearance of fat and regeneration. In 5 out of 7 patients, there was an increase in the mesenchymal reaction. One might consider that the increase in fibrosis seen in our material following lipotropic therapy was due to a collapse of the reticulum as a result of loss of fat from liver cells. There are, however, several points which are in favor of the increase in fibrosis being reactive and not due to collapse of the reticulum. First, paralleling the increase in connective tissue there is also an accompanying increase in periportal infiltration. Second, specific fiber stains reveal definite changes in the reticulum pattern. The disappearance of fat without decrease of fibrosis after lipotropic therapy agrees with the findings of Gilman⁶ and Beams² and the clinical observations of Weir²⁰ and others¹ in human cirrhosis. Recently, Best's group of workers¹⁹ has shown in rats, an apparent decrease in fibrosis after lipotropic therapy. This discrepancy between human and animal experiments may possibly be due to species differences in the handling of fat. Time as a factor, especially in view of the shorter life span of the experimental animal as compared to man, also must be taken into consideration in that our longest period of histologic checkup was seven months. It is possible that with longer periods of observation, a more favorable connective tissue response to lipotropic agents might have been obtained. Nevertheless, the clinical implications of progressive fibrosis found histologically in the liver in some patients, coincident with clinical and functional improvement, is important in the evaluation of cirrhosis therapy. The fact that dietary and lipotropic therapy of cirrhosis has undoubtedly been beneficial from both a clinical and laboratory standpoint does not necessarily mean that such therapy can bring about cure of this chronic disease. It is possible that many of the patients who have shown marked improvement under lipotropic therapy, if followed for a long enough period of time, would be found eventually to have succumbed as a result of the continued fibrotic process in the liver. The stage of irreversibility in the liver in some patients may occur quite early even in the fatty stage of cirrhosis. These facts deserve consideration in the evaluation of lipotropic therapy of cirrhosis.

The clinical improvement noted in all our patients is in keeping with experiences of others^{1, 2, 6, 11, 15, 17} and needs no further comment. Likewise, as was expected, functional improvement paralleled the clinical response.

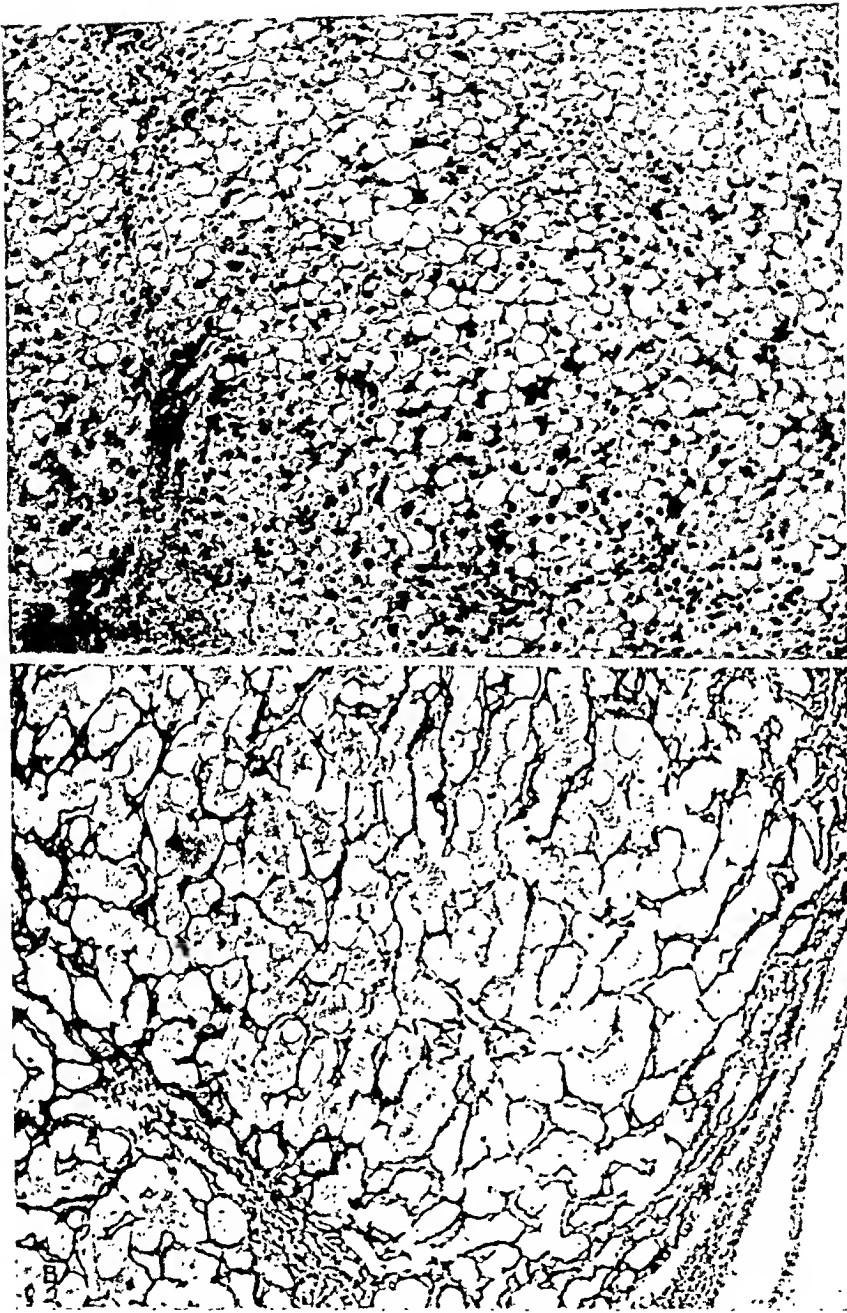


FIG. 2. Liver biopsy specimen of a patient with early portal cirrhosis before treatment: (A) Hematoxylin eosin stain. The normal lobular architecture is missing. The liver cells of the nodules reveal extensive fatty metamorphosis. The connective tissue trabeculae are of moderate width and reveal a scattered infiltration of lymphocytes and histiocytes almost without admixture of polymorphonuclear leukocytes. The border zone between parenchyma and trabeculae is sharply demarcated. (B) Gomori's reticulum fiber stain. The fibers in the nodules are separated by the large fat-containing cells. The trabeculae reveal a moderately dense structure.

The striking increase in serum cholesterol following lipotropic therapy is consistent with Bloor's³ findings and hypothesis that cholesterol, as well as phospholipids may play an important role in fatty acid transport. Lack of decrease in the thymol turbidity after therapy, may have several possible explanations. It may be due to regeneration still taking place in the liver. Hoagland¹² has postulated that the thymol turbidity may be a measure of regeneration rather than of parenchymal damage. Another possible explanation may be the hypercholesterolemia following therapy noted in this study. It has been demonstrated that thymol turbidity depends not only on globulin but also bears some relation to the serum lipids.^{5, 18} In some types of lipemia the thymol turbidity may be increased independent of liver damage.

SUMMARY

The morphologic, functional and clinical responses to different forms of "lipotropic" therapy (cystine plus choline, methionine and high protein-high vitamin B complex diet) were studied in 15 patients with fatty livers (10 fatty cirrhosis, 5 "nutritional" fatty livers).

Clinical improvement, as evidenced by subjective and objective findings, was noted in almost all patients after "lipotropic" therapy.

Functional improvement was evident in all tests performed except in the thymol turbidity and total cholesterol which remained abnormal.

Disappearance of fat and regeneration of parenchymal cells were noted in all instances after "lipotropic" therapy. In the cirrhotic group the inflammatory and fibrosing reaction especially in the portal triads were more marked after therapy in 5 out of 7 patients in whom biopsies were repeated.

The significance of the findings and their implications in the evaluation of "lipotropic" therapy are discussed.

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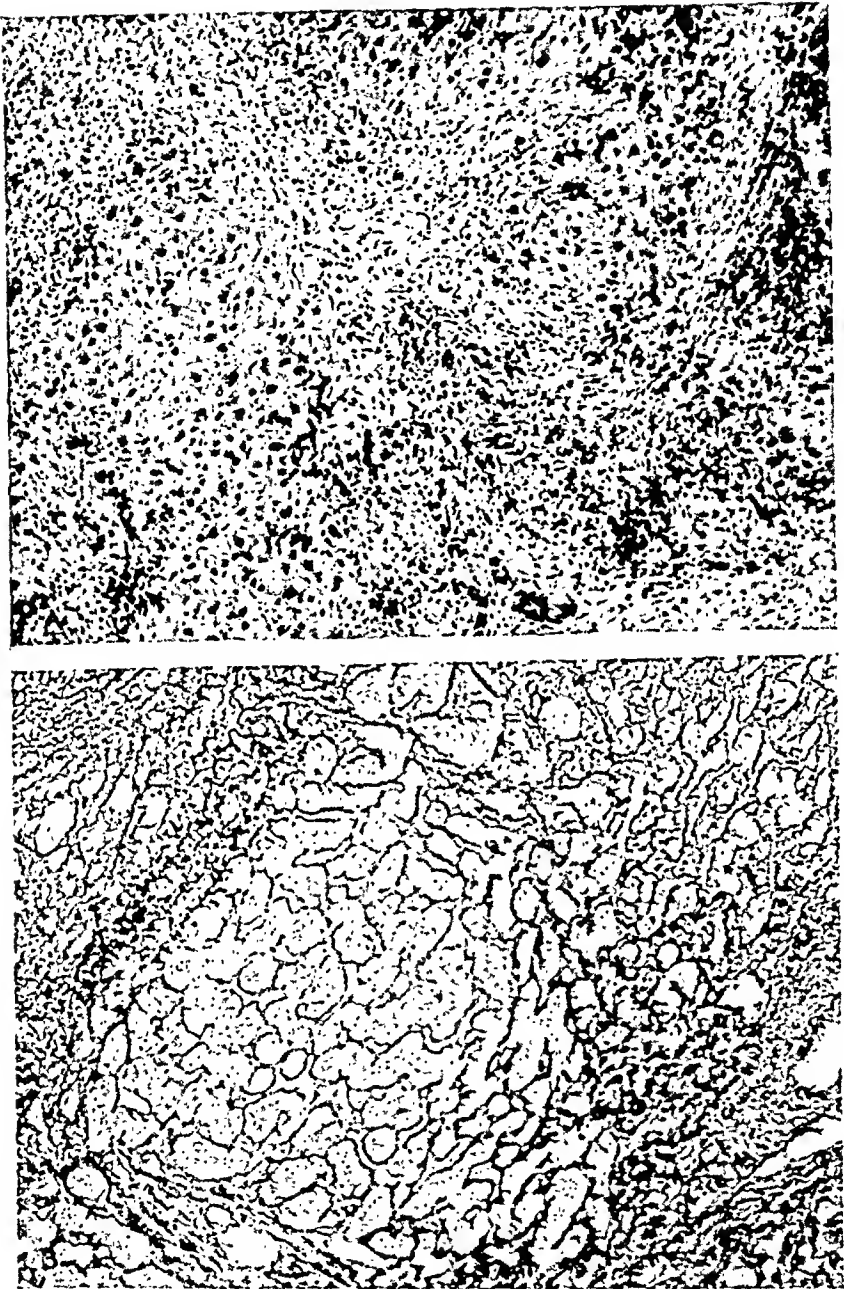


FIG. 3. Liver biopsy specimen of patient (Fig. 2) taken four weeks after treatment with methionine. (A) Hematoxylin eosin stain. The liver cells are almost free of fat. The cytoplasm of the liver cells varies markedly in size and staining quality. In the periphery of the nodules, marked regeneration is noted. The wide connective tissue trabeculae reveal marked cellular infiltration with lymphocytes, histiocytes and polymorphonuclear cells. The trabeculae radiate into the surrounding parenchyma demarcating the nodules poorly. (B) Gomori's reticulum fiber stain. The reticulum fibers in the nodules are more closely spaced than in Figure 2B. The texture of the trabeculae appears denser and sprouting of fibers is noted, suggesting proliferation.

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EVALUATION OF PAPANICOLAOU'S METHOD OF CANCER DIAGNOSIS*

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While Papanicolaou¹¹ recognized cancer cells in vaginal smears as early as 1928, his diagnostic method did not become widely known until fifteen years later following publication of his and Traut's¹² beautiful atlas entitled, *Diagnosis of Uterine Cancer by the Vaginal Smear*.

Meigs,¹⁰ Ayre,¹ Jones and coworkers⁶ accepted this method enthusiastically and recommended it as a part of every routine physical examination in office, outpatient department and hospital. Meigs¹⁰ urged State Boards of Health to make a diagnostic service by smears available to all physicians of the community, and several State Board of Health laboratories followed his advice.

Since, after all, it is the practicing pathologist who has to determine the usefulness of the test, it is surprising that only one paper evaluating Papanicolaou's method has come from a pathologic laboratory thus far. In 1945, Gates and Warren³ reported their results on 341 vaginal smears, but were unable to arrive at a definite conclusion. They felt that the procedure, while highly promising, had yet to be clearly established as a means of final diagnosis and that there had to be more specific information on the limitations and advantages of the method before it could be accepted as a routine test.

TECHNIC

Since January 1947, Papanicolaou's method has been used in our laboratory in more than 100 cases from the gynecologic service of St. Francis Hospital and the Sedgwick County Tumor Clinic. The specimens were obtained and stained by one of us (J. B. W.). The procedure of taking vaginal smears was that described by Papanicolaou.¹³

A slightly curved glass pipet, 6 inches long and 0.5 cm. in diameter, was attached to a 3 inch rubber suction bulb. The pipet was dry and the patient was advised not to douche on the same day, since water in the vaginal smear interferes with cellular detail. The glass pipet was introduced without a speculum into the posterior fornix of the vagina while the bulb was compressed. The bulb was released and the pipet was withdrawn slowly. The material thus obtained was blown on a previously marked slide and a thin spread was obtained, either with the convex side of the pipet, or by using a second slide as is done in blood slides. Thick smears do not give a satisfactory cell picture. The slides were immediately fixed in a solution of equal parts of 95 per cent alcohol and

* Presented at the Twenty-Sixth Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 28, 1947. Received for publication, October 13, 1947.

ether. Drying of the smears should be avoided. Fixation for five minutes is sufficient but slides may be left in the fixative for as long as two weeks.

Papanicolaou, in his atlas, gives instructions as to how patients may prepare their own smears "when a larger number of them is required for study". He finds that vaginal smears prepared by the patients are, as a rule, satisfactory. This advice seems to us unsound because of our own experience and because the article by Gates and Warren³ stated that 25 per cent of the slides taken in Meigs' clinic were technically unsuitable. Since thin even smears are essential for a satisfactory study, the physician or a well trained technician should make the smear. As a routine, we made three smears in each instance, two of which were stained with Papanicolaou's trichrome method and the third with Harris' hematoxylin and eosin. The results with the latter method compared very well to results with the stain recommended by Papanicolaou, since both procedures use the same nuclear stain, *i.e.*, Harris' hematoxylin, and the cytologic diagnosis depends entirely on nuclear characteristics and not on the polychrome stain of the cytoplasm.

Staining Procedure

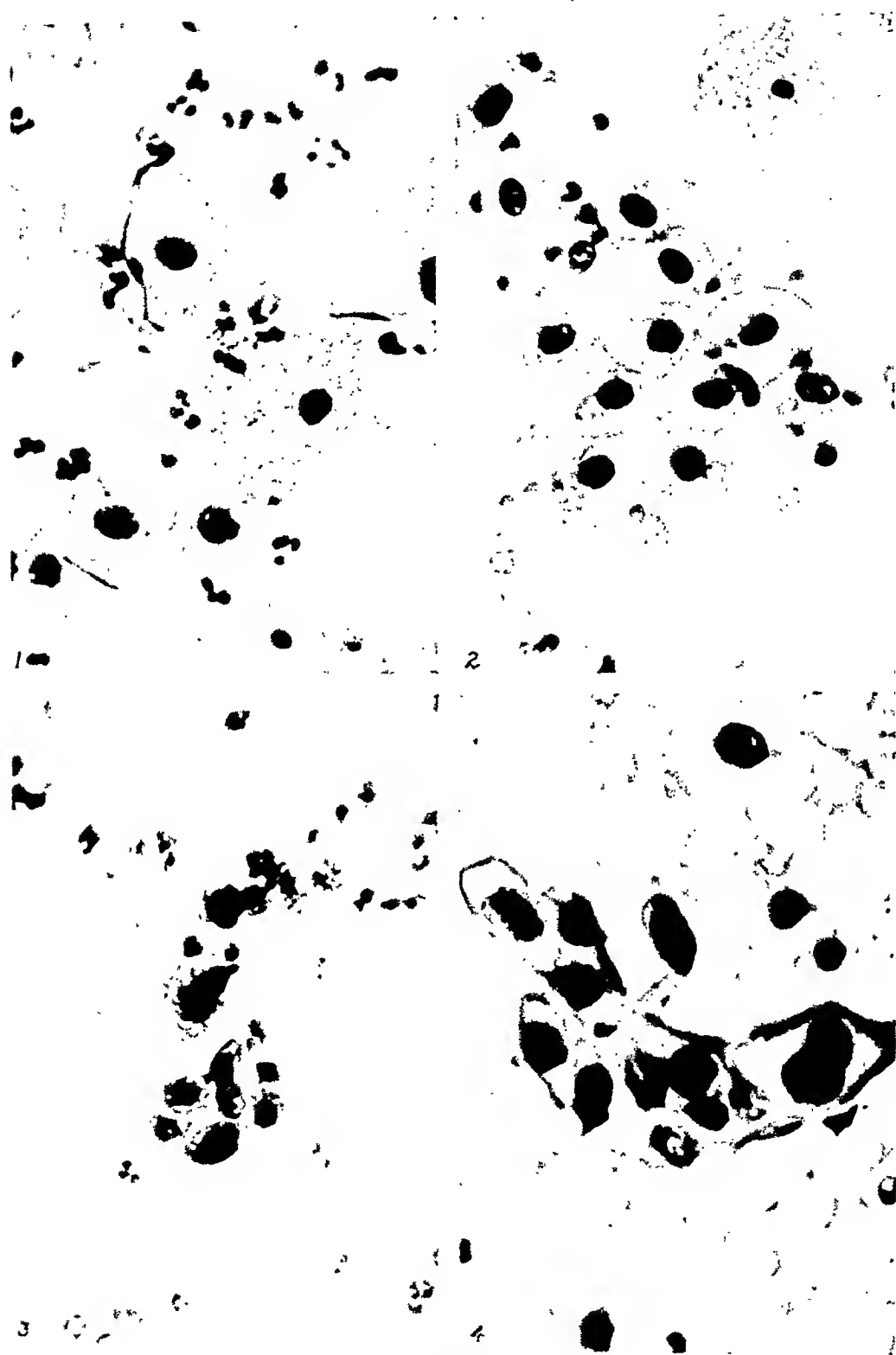
1. After fixation for five minutes or longer in alcohol-ether, rinse the slides successively in 70 per cent alcohol, 50 per cent alcohol and distilled water.
 2. Stain in Harris' hematoxylin for five minutes.
 3. Decolorize in 0.5 per cent aqueous hydrochloric acid solution.
 4. Wash in running water for seven minutes and rinse in distilled water.
 5. Run successively through 50, 70, 80 and 95 per cent alcohol.
 6. Stain for one minute in orange G-6.
 7. Rinse five to ten times in each of two jars containing 95 per cent alcohol to remove excess stain.
 8. Stain in EA-50 for two minutes.
 9. Rinse five to ten times in each of three jars containing fresh 95 per cent alcohol.
 10. Dehydrate in absolute alcohol, clear in xylol and mount in Canada balsam.
- Staining reaction.* Nuclei are dark purple, basophilic cytoplasm is green, acidophilic cytoplasm is pink to orange and totally cornified cells are orange or yellow.

The two counterstains OG-6 and EA-50 can be obtained in solution from Ortho Pharmaccutical Corporation, Linden, New Jersey, or are easily prepared from the formula given by Papanicolaou in his atlas.

Orange G-6

Orange G, 0.5 per cent alcoholic solution	100 cc.
Acid phosphotungstic	0.015 Gm.

- FIG. 1. Normal cornified cells with abundant cytoplasm and small dark nuclei.
X 480
- FIG. 2. Normal basal cells with uniform nuclei. X 480
- FIG. 3. Endometrial cells from benign glandular hyperplasia. X 480
- FIG. 4. Group of atypical cells from squamous cell carcinoma of cervix. X 480



FIGS. 1-4

EA-36

Light green SF yellowish—0.5 per cent solution in 95 per cent alcohol	45 cc.
Bismarck brown—0.5 per cent solution in 95 per cent alcohol	12 cc.
Eosin yellowish—0.5 per cent solution in 95 per cent alcohol	45 cc.
Acid phosphotungstic	0.2 Gm.
Lithium carbonate saturated aqueous solution	1 drop

Note. The 0.5 per cent alcoholic solutions are first prepared using heat, since the solubility of the stains in 95 per cent alcohol is low. The solutions are filtered separately, just before mixing them for the preparation of the final stain.

No special equipment or technical personnel is necessary if Harris' hematoxylin and eosin is used. The smears, after fixation in alcohol-ether, are run with the daily batch of paraffin sections through hematoxylin, hydrochloric acid, running water, eosin, water, alcohol, xylol and Canada balsam. If care is taken to avoid overstaining with eosin, the cytologic diagnosis is no more difficult on those slides than on those treated with the trichrome stain. By substituting Papanicolaou's stain with the simple hematoxylin-eosin method, even the busiest laboratory will be able to become familiar with the diagnostic method of Papanicolaou.

RESULTS

While we have studied more than 300 smears during the last nine months, only 70 gynecologic cases form the basis of this analysis, because the results in these cases could be satisfactorily compared with the diagnoses obtained by tissue examination.

From vaginal smears, 44 cases were diagnosed as negative, 23 as positive and 3 as suspicious. Three of the smears (7 per cent), which were considered to be negative, were incorrect, since subsequent biopsy or curettage revealed carcinoma of the cervix in one patient and endometrial adenocarcinoma in 2 patients. Of the 23 smears considered to be positive, 2 (9 per cent) were incorrect. By curettage and biopsy and on careful clinical follow-up study, no malignancy was revealed in these 2 patients. Likewise, in the 2 cases with suspicious smears, anatomic and clinical findings did not suggest cancer.

DISCUSSION

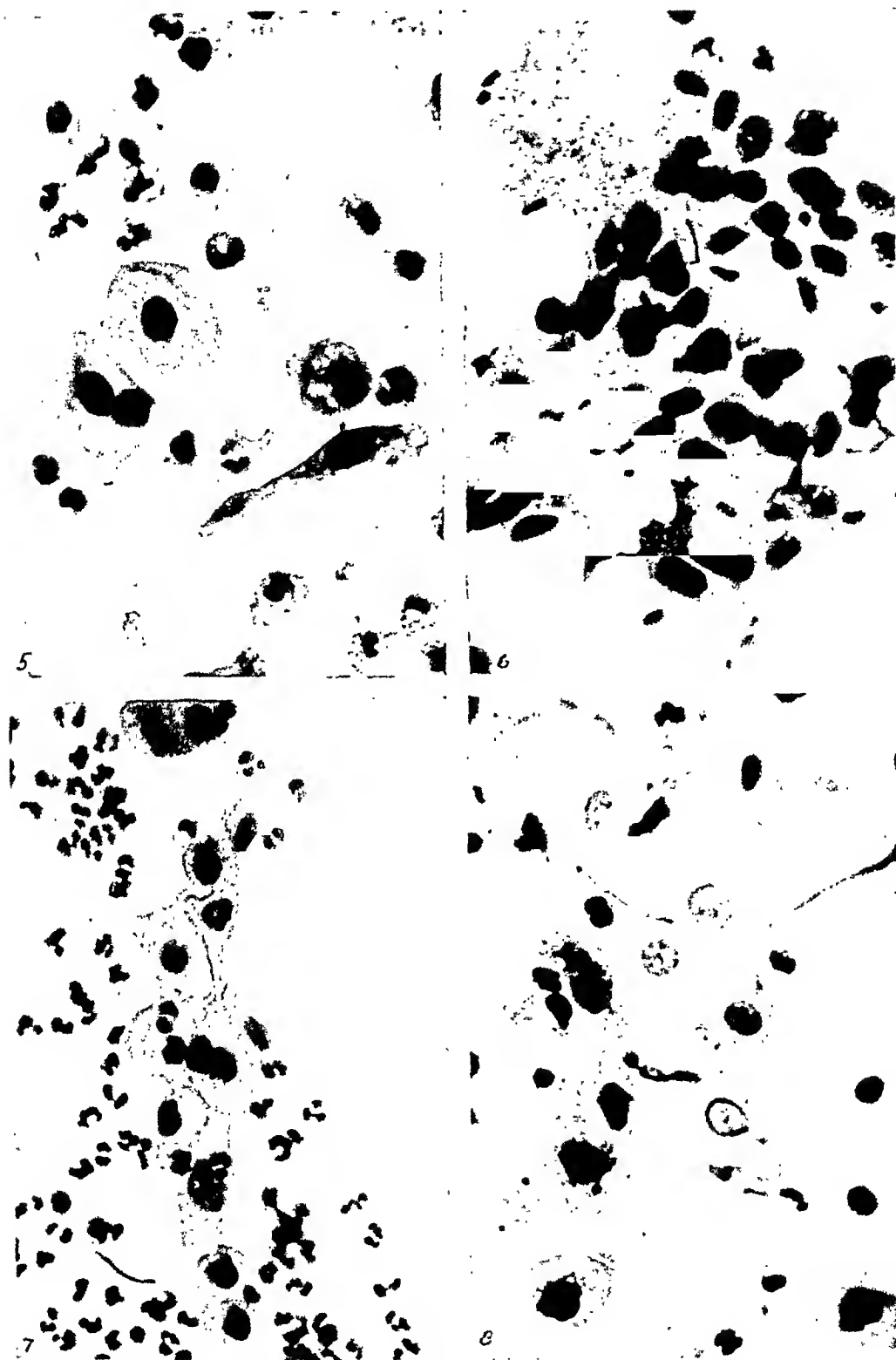
Gynecologic clinics have reported a high reliability of the vaginal smear diagnosis. Meigs and coworkers,¹⁰ in 153 noncancerous cases, had only 2.6 per cent

FIG. 5. Squamous cell carcinoma. Note marked variation in size and form of cells. $\times 480$

FIG. 6. Irradiated squamous cell carcinoma. Sheet of atypical cells with hyperchromatic nuclei. $\times 480$

FIG. 7. Atypical cells from adenocarcinoma of endometrium. $\times 480$

FIG. 8. Vacuolated atypical cells from endometrial adenocarcinoma. $\times 480$



FIGS. 5-8

positive smears, while Papanicolaou and Traut¹³ mentioned no false-positive diagnoses in their series. Ayre¹ had 8 per cent false-positive or suspicious smears, while Jones and coworkers⁶ reported 16 false-positive diagnoses in 375 noncancerous patients.

In a series of 39 endometrial and 109 cervical cancers observed by Meigs, 16 smears (10.3 per cent) were incorrectly called negative by vaginal smear. Of these, 8 (20 per cent) were from endometrial and 8 (7.4 per cent) from cervical cancer cases. Ayre reported that 38 of his 40 patients with carcinoma of the uterus showed neoplastic cells in the vaginal smear, while Jones had 7 (21 per cent) false-negative diagnoses by smear in 33 cases of endometrial adenocarcinoma.

The high degree of accuracy reported by these authors has been accomplished apparently not by a single examination, but more likely by a number of smears repeated at different times in a given case. Meigs,¹⁰ for instance, made the statement that only 6 of 16 successive smears taken in a period of seven days from a woman known to have cancer, showed cancer cells. Gates and Warren³ gave their interpretation of each submitted smear on pathologic criteria only and were not in possession of all the clinical data as were the clinical observers. As a result, they present a much less favorable picture in respect to the accuracy of Papanicolaou's diagnostic method.

Of the total of 123 smears from 101 patients with clinical or pathologic evidence of malignant disease in the genital tract there were one false-positive and 8 doubtful smears (8 per cent). In 12 of 38 patients (37 per cent) with nonirradiated malignancies there was lack of agreement in diagnosis from smear and clinical and pathologic data. There were discrepancies in diagnoses as made from smear and tissue section in 47 of 173 patients (21 per cent) with postirradiation carcinoma. Gates and Warren felt that smears from highly malignant carcinomas of the cervix, some adenocarcinomas of the cervix, as well as irradiated carcinomas, present very difficult problems for the cytologist.

THE "SINGLE CELL" DIAGNOSIS OF CANCER

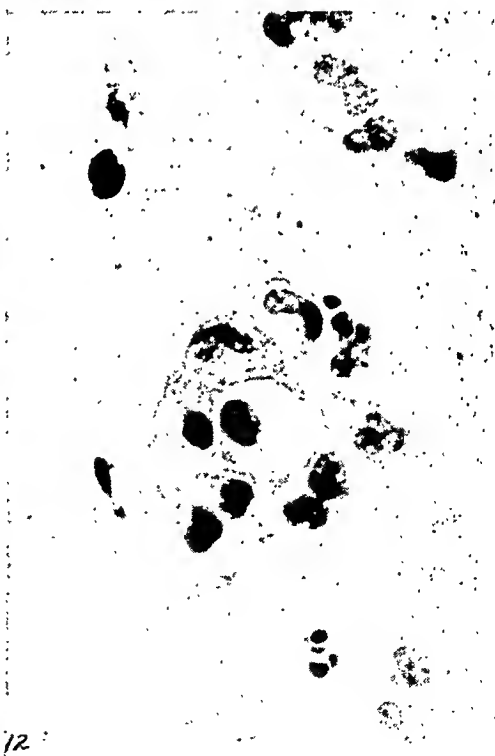
The characteristics of the malignant cell have been studied since the unsuccessful attempts of Lebert⁸ and Hannover⁵ in the middle of the last century to characterize morphologically the "specific" cancer cell. The best known of recent attempts to diagnose cancer from the morphology of a "single cell" is that of MacCarty.⁹ Examining fresh frozen sections under oil immersion, he recognized a malignant cell by its relatively large nucleolus. This diagnostic sign has been refuted by Guttman and Halpern⁴ who made many actual measurements

FIG. 9. Row of atypical cells from adenocarcinoma of endometrium. $\times 480$

FIG. 10. Immature cells of cervical erosion. Amputated cervix did not reveal malignant changes. $\times 480$

FIG. 11. Sheet of vaginal cells with prominent nucleoli. No clinical evidence of carcinoma. Hysterectomy three years previously for myosarcoma of uterus. Postoperative irradiation. $\times 480$.

FIG. 12. Groups of atypical cells from patient 63 years of age. Curettage revealed benign uterine polyp and benign endometrium. $\times 480$.



FIGS. 9-12

on benign and malignant tumors and on normal tissue. These authors pointed out that nucleoli may be larger in simple hyperplastic tissues than in malignant tumors and they concluded that the quantitative differences between nuclear-nucleolar ratios of normal tissue, hyperplastic tissue, benign and malignant tumors were not significant.

The cytologic diagnosis of exudates from body cavities has been practiced for many years, but the accuracy of diagnosis has never been remarkable (Foot³ 68 per cent). It is of interest to note that MacCarty refused to apply his "single cell diagnosis" to exudates from body cavities. In a recent article on specificity of cancer tissue, E. Knake⁷ came to the conclusion that there is no morphologic characteristic known today which differentiates a malignant from a noncancerous cell.

It should be emphasized that the trichrome stain introduced by Papanicolaou is not a specific stain for cancer cells, as many physicians seem to believe. The only advantage of this staining procedure results from the light staining of the cytoplasm which preserves the translucency of the smear. In his latest publication, Papanicolaou points out that the nuclear changes of cancer cells offer the most reliable diagnostic criteria. Anisocytosis and anisonucleosis are often marked. Many nuclei are hyperchromatic or show fragmentation. Mitoses are rare. In certain types of cancer the nucleoli are very conspicuous. In cervical carcinoma one often finds abnormal and bizarre forms, many cells become elongated or take on a shape resembling a tadpole. Papanicolaou's description of the cellular changes in cancer does not add anything new to their morphology and the pictures in Papanicolaou's atlas, admirable as their rendition may be, are familiar to the tumor pathologist, as he encounters such changes in sections of degenerated or poorly preserved tissue.

In this connection it is well to remember that former attempts to diagnose uterine cancer from smears of cells have not stood the test of time. Babes,² in 1928, recommended cell smears in suspected cancer of the cervix to avoid the supposed danger of biopsy. He took the material with a stiff platinum loop from the suspicious lesion, fixed the smear with alcohol and stained it with hematoxylin and eosin. His diagnoses were based on the atypical character of the epithelial cells and proved to be correct in 18 of 20 cases of cancer of the cervix. Schiller¹⁴ also recommended scraping the surface of suspicious cervical lesions for a cytologic diagnosis and R. Meyer² conceded that this procedure may often furnish sufficient material on which to arrive at a positive diagnosis, but he questioned its general usefulness. Schiller's method will fail in some cases because cancer cells may be well differentiated and of almost typical cell structure, while in healing erosions immature cells of a very suspicious appearance may be found. Mitoses and especially irregular forms of nuclei are often missed in young stages of carcinoma of the cervix.

Papanicolaou and Traut¹³ maintain that the vaginal smear is the only practical method thus far developed which is useful in revealing the very early carcinomatous processes of the cervix. Our knowledge in regard to "early carcinoma",

precancer and noninvasive cancer is still limited and a conservative attitude seems warranted. There is some evidence that carcinoma *in situ* of the cervix is a very slow process comparable to Bowen's disease of the skin (Schiller).¹⁴ The fact that it is impossible from the smear to differentiate between precancerous changes, carcinoma *in situ* and invasive carcinoma is one of the greatest disadvantages of Papanicolaou's method. We agree with Gates and Warren³ that the diagnosis and treatment of "early carcinoma" should not be lightly undertaken, since many of the patients are in the childbearing age and some are very young.

In irradiated tissue fibroblasts and epithelial cells may acquire all the morphologic features of malignant cells and yet remain benign. These atypical cell structures are readily distinguished from cancer in tissue sections but not so in smears. The isolated irradiated cells may closely resemble malignant cells. The same atypical features may be seen in cervicitis, especially in older women. The two false-positive and two false suspicious smears in our series were either from patients following irradiation or from elderly patients with uterine polyps.

SUMMARY

Clinical pathologists should give Papanicolaou's method a trial, formulate its advantages and limitations and establish the place of this method in the diagnosis of cancer. In our laboratory the method has been used for nine months with an average accuracy of 80 per cent. Papanicolaou's method has a definite, but limited, usefulness wherever biopsy or curettage is not feasible. In instances where a suspicious lesion is accessible to biopsy or curettage, the vaginal smear method is not indicated.

By the vaginal smear it is not possible to differentiate between changes due to irradiation, carcinoma *in situ* and invasive carcinoma. Therefore, tissue examination never can be replaced by vaginal smears in the decisive diagnosis of cancer.

The high percentage of false-negative smears in endometrial carcinoma (20 per cent) counts heavily against the use of vaginal smears as a screening test in cancer detection clinics.

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USE OF WRIGHT'S STAIN IN DIAGNOSIS OF MALIGNANT CELLS IN BRONCHIAL ASPIRATIONS*

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In the diagnosis of malignancy by means of the examination of cells in exudates and body fluids the emphasis has been placed on the use of block sections or of Papanicolaou or other special stains. Little attention has been given to the possibilities of Wright's stain in bronchial secretions, although this stain has stood the test of time as being capable of revealing fine differences in nuclear and cytoplasmic structures in blood and bone marrow smears and in imprints from lymph nodes. In this paper the morphology of malignant cells in material aspirated from the bronchi and stained by the ordinary Wright's stain is presented.

METHODS

The material from the bronchi was collected at the time of bronchoscopy by suction into a stoppered test tube having two side arms. From 1 to 5 cc. of fluid was collected from the area most likely to reveal abnormality as determined by clinical signs and symptoms, by roentgenograms and by direct bronchoscopic observations. The material was sent immediately to the laboratory and processed as soon as possible.

The procedure used in making smears varied with the character of the fluid received. If the fluid was grossly bloody, it was mixed by means of a bulb pipet. Small drops were placed on a clean slide and smears were made using a cover slip as a spreader. If, as often was the case, the fluid was clotted, the clot was vigorously agitated and smears were made of the defibrinated fluid. Six or more smears were made. Half of these were allowed to dry in the air, and the others allowed to dry partly, then fixed for twenty minutes or more in a mixture containing equal parts of 95 per cent alcohol and ether.

If the fluid contained little blood and was thin and watery due to dilution with saline, the material was centrifuged in a conical tube, the supernatant fluid discarded and approximately 1 cc. of human blood serum added. The sediment was mixed with the serum and again centrifuged, after which smears were made of the cellular portion. The serum added to the sediment aids in fixation and tends to decrease the crystallization of salts which occurs when the slides dry.

Flecks of tissue or cell aggregates were aspirated by means of a capillary pipet, placed on a slide and another slide pressed down on the cell mass. The squashed material was spread by pulling the two slides apart and the smears fixed in alcohol and ether. Smears placed in fixative were allowed to dry before being stained.

* Presented at the Twenty-Sixth Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 28, 1947. Received for publication, October 28, 1947.

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The dried smears were first completely covered with Wright's stain for one minute. Phosphate buffer solution (6.63 Gm. of KH_2PO_4 , and 3.2 Gm. of $\text{Na}_2\text{HPO}_4 \cdot 2 \text{H}_2\text{O}$, in 1000 ml. of distilled water) was then added followed by distilled water. The number of drops of buffer solution and distilled water to give ideal staining properties was determined by trial and error with each batch of Wright's stain. The diluted stain was left on from six to eight minutes, after which the slide was washed with distilled water, drained and dried in the air.

Each smear was systematically searched under low power. Suspicious individual cells or cell groups were examined under oil immersion. The examination usually required several hours, the exception being the rare case of malignancy in which there were numerous unmistakable groups of malignant cells.

In this study, 90 specimens were examined of which 29 were shown by bronchial biopsy, operation or autopsy to be malignant. At the beginning of the study, stains by Papanicolaou's method were made along with the Wright's stains, but after examining the preparations made by the two technics in approximately 50 cases, Papanicolaou's method was discontinued, for it did not reveal any structure which could not be readily demonstrated by means of Wright's method.

MORPHOLOGY OF MALIGNANT CELLS

The morphologic characteristics of malignant cells which have been described by numerous authors are revealed in the cells of bronchial secretions when treated by Wright's stain.

With Wright's stain, the undifferentiated and immature malignant cells, whether of mesodermal or epithelial origin, have the same general characteristics as do immature cells of the leukemias or plasma cell myeloma. These cells usually are large, have a relatively large nucleus and little cytoplasm, which tends to take the basic stain. The nuclei of the undifferentiated cells are predominantly acidophilic; the chromatin structures are delicate and well defined and nucleoli are, as a rule, demonstrable. The large size of the cells, the prominent nuclei and the basophilic staining of the cytoplasm make the malignant cells stand out prominently against the lighter background.

Although exfoliated malignant cells are often single and reveal striking abnormalities (Fig. 1), abnormal cell groups are of greater diagnostic value. In nonmalignant groups, the cells are characterized by uniformity in size, shape and staining. The nuclei of nonmalignant groups are generally round or oval and have an orderly chromatin structure. In malignancy, the individual cells show marked variation in size, shape and nuclear structure (Figs. 2, 3, 4, 5C,

FIG. 1. Cells in bronchial secretion from patients with proved lung malignancy. Wright's stain. $\times 1000$.

- A. Large cell with relatively large irregular nucleus. From an undifferentiated carcinoma.
- B. Large cell with degenerative nucleus. From a squamous cell carcinoma.
- C. Extremely large cell with bluish-staining material attached to cytoplasm. From an epidermoid carcinoma.
- D. Giant cell with large multilobulated nucleus and prominent nucleoli. From an undifferentiated carcinoma.

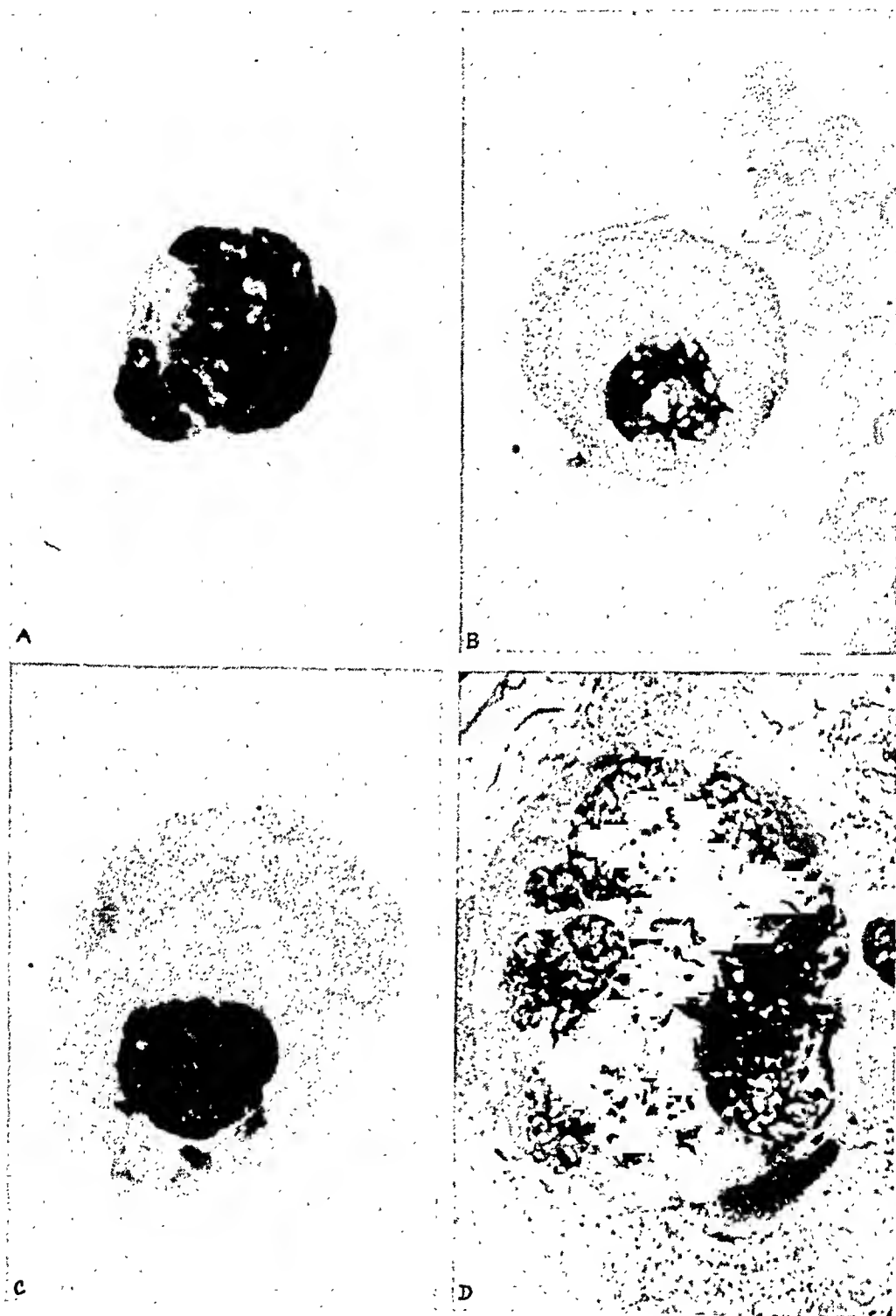


FIG. 1

5D, 6D and 6F). Malignant cells are often compressed and have a disorderedly arrangement. Different cell groups in the same preparation do not look alike (Fig. 2). Organoid arrangement of epithelial cells is occasionally observed.

The nuclear structure in malignant cells reveals varied abnormalities. Instead of the chromatin being arranged in a symmetrical and orderly manner, there is irregularity in the structural pattern. There may be an increase or a decrease in the amount of chromatin material. The nuclear membrane is often ill-defined and the chromatin material seems to be scattered within the cytoplasm (Figs. 4D, 6A).

Malignancy is characterized by bizarre and lobulated nuclei and by multiple nuclei within a given cell (Figs. 1A, 1D, 4D, 6A, 6B, 6C, 6E and 6F). Uneven numbers of nuclei and variations in structure in different nuclei within the same cell are suggestive of malignancy. Sometimes one finds within a given giant cell, not only multiple nuclei, but cells within cells or localized areas of keratinization (Fig. 6C). Multiple nucleoli may be demonstrable (Fig. 1D).

The nucleoli in cells stained by Wright's method appear as relatively light hyaline areas taking a purplish stain. Dark dots or larger dense areas within the nucleus are to be interpreted as evidence of degeneration and not as nucleoli (Figs. 2C, 4B). Nucleoli in nonmalignant cells as a rule are quite small, whereas in malignancy they may be as large as red blood cells and may occupy a disproportionately large area within the nucleus.

Mitotic figures and evidence of cell division are of value in making the diagnosis in malignancy, but in bronchial exudates from patients with known cancer, evidences of mitosis are the exception rather than the rule.

Vacuoles and relatively clear spaces within the cytoplasm appear in both malignant and nonmalignant cells. Signet ring forms are seldom seen and are not diagnostic when found. Phagocytosis of brown or black pigment granules or red blood cells and bacteria or other extraneous material within the cytoplasm are evidence that the cell is not malignant. Cilia attached to cells are indicative of a degree of differentiation that does not occur in tumors.

Well-defined, bluish masses having a foamy structure are attached to some epithelial cells (Figs. 1C, 1D, 2A, 6B). The position and morphology of this material suggests intercellular elements. These attachments, although prominent in some cases of malignancy, are not specific.

DISCUSSION

In addition to the ability of Wright's stain to reveal significant morphologic changes, the other advantages of the Wright's stain are that it is readily available, technicians and pathologists are familiar with its use and staining properties and there is daily control over reagents used. The technic is relatively

FIG. 2. Malignant cells in bronchial secretion from patient with undifferentiated carcinoma of lung showing variability in size, shape and staining reaction of cells and in nuclear shape and structure. Wright's stain. $\times 1000$.

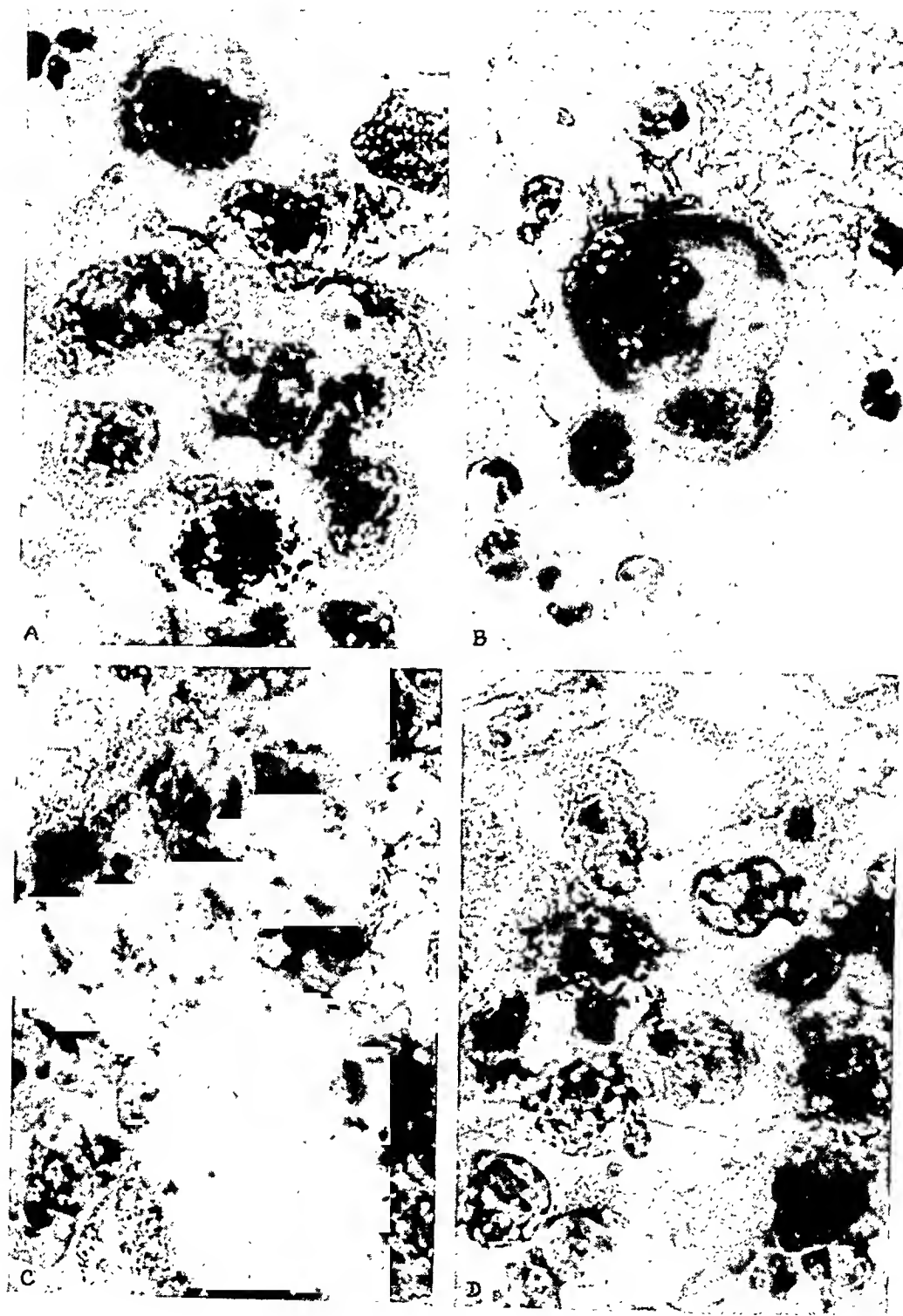


FIG. 2

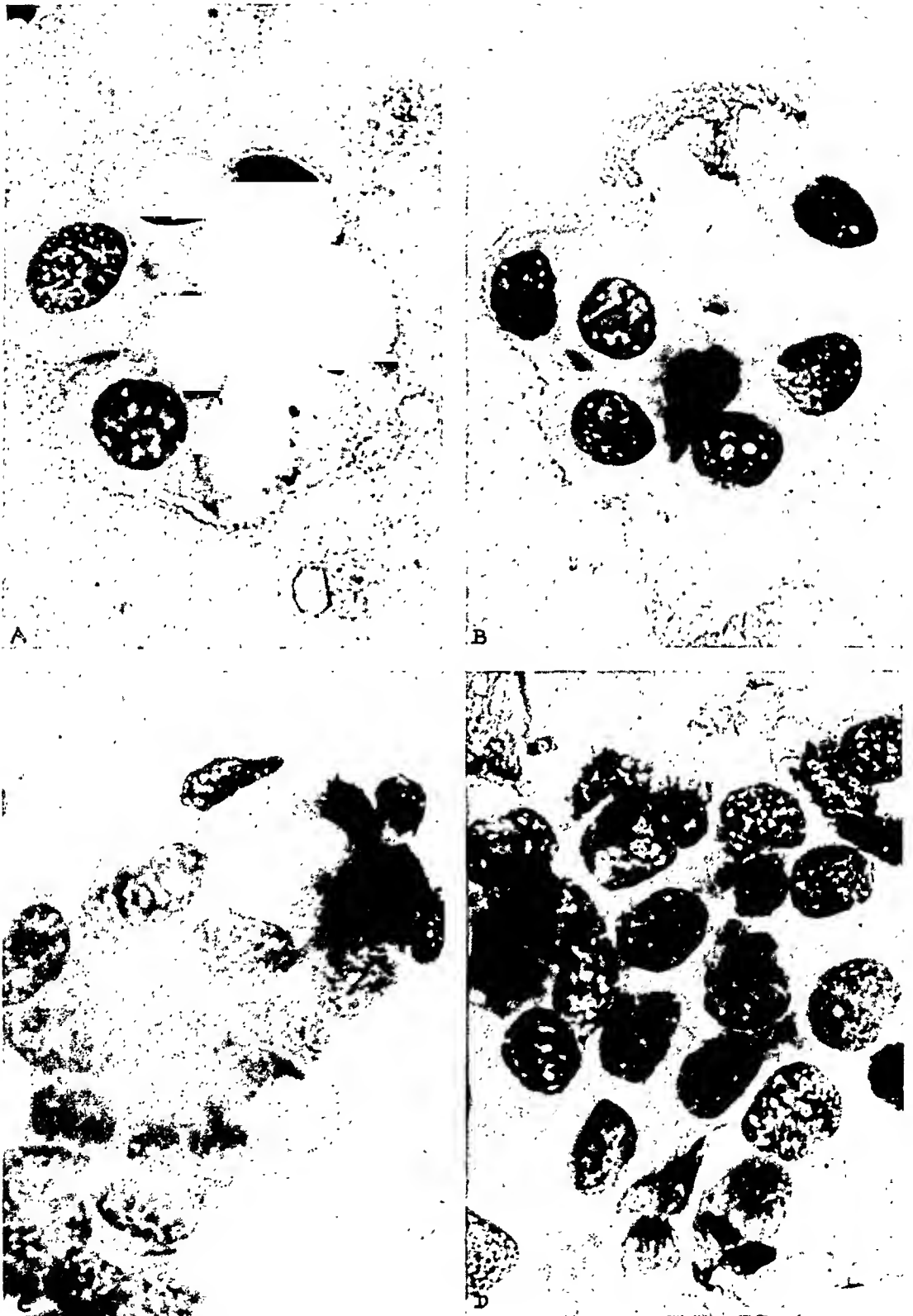


FIG. 3. Grouped cells in bronchial secretion from patients with proved lung malignancy. Wright's stain. $\times 1000$

- A. From a small (reserve) cell carcinoma showing variation in color of cytoplasm and nuclear structure.
- B. From a squamous cell carcinoma.
- C. From an undifferentiated carcinoma with cells irregularly arranged.
- D. From a small cell carcinoma.

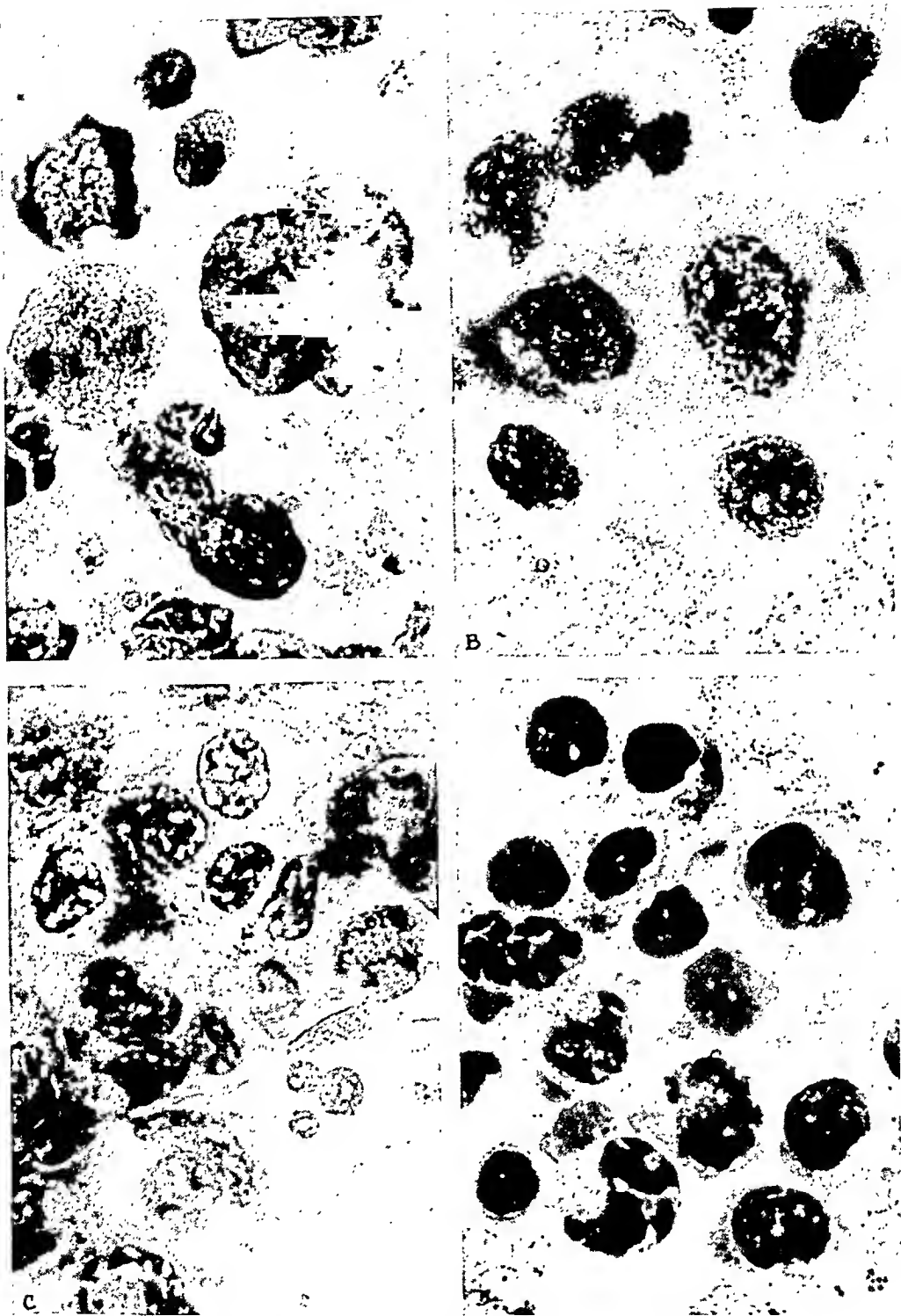
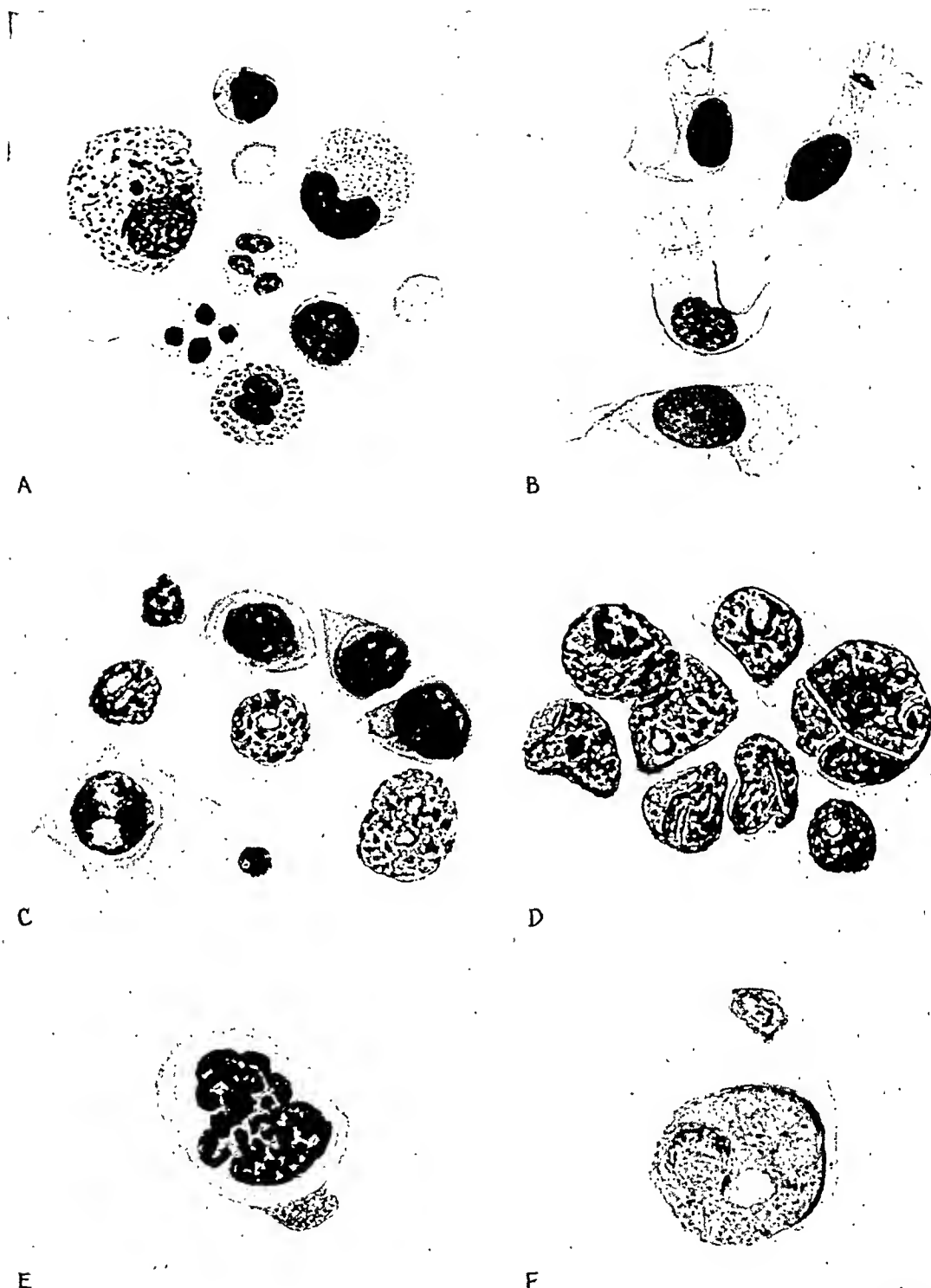


FIG. 4. Cell groups in bronchial secretion from patients with proved lung malignancy. Wright's stain. $\times 1000$

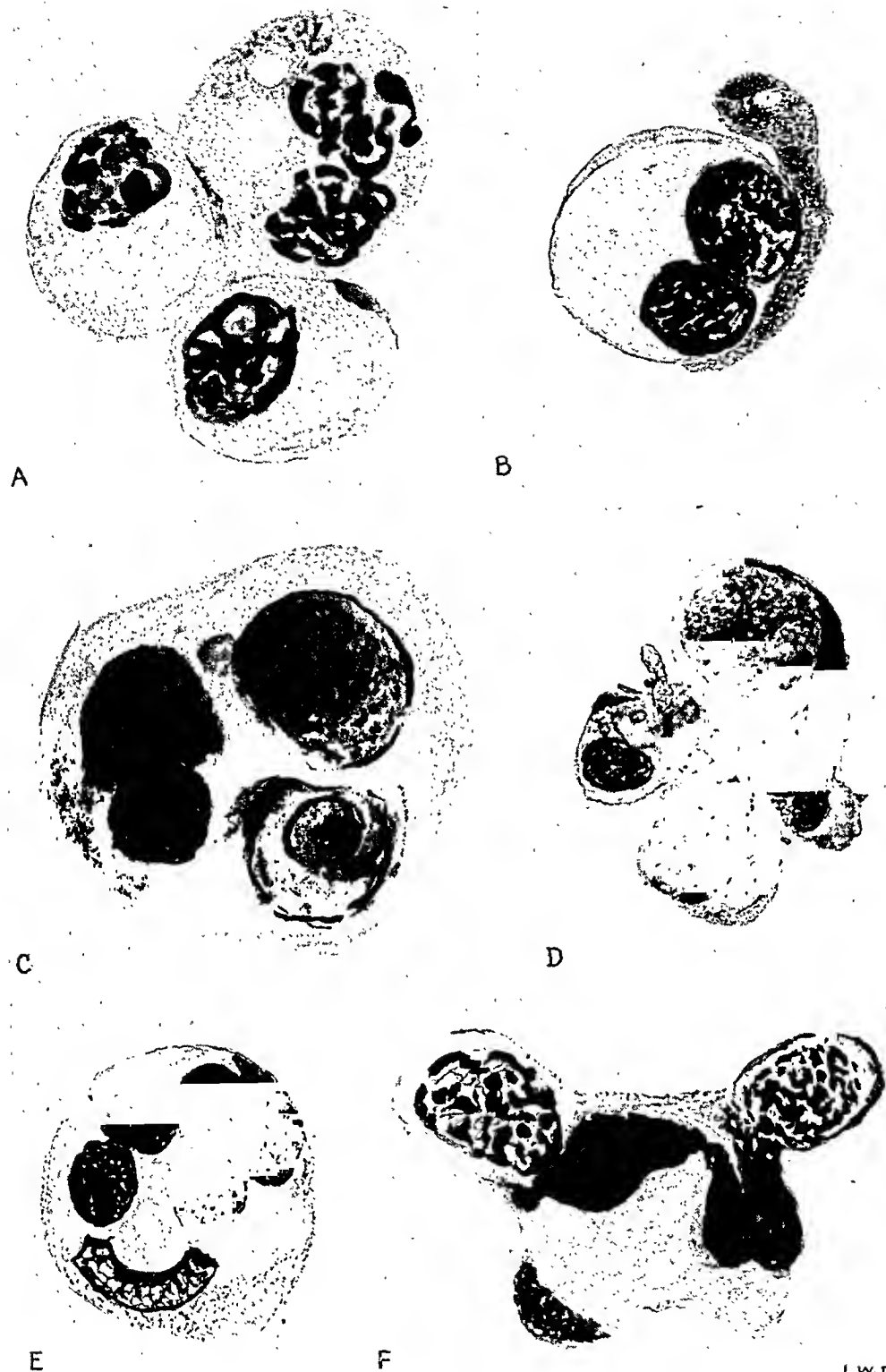
- A. Pleomorphism in cell types. Early nuclei with nucleoli from a metastatic carcinoma.
- B. Large irregular cells with variation in nuclear pattern from a metastatic carcinoma.
- C. Pleomorphism from a metastatic carcinoma.
- D. From a moderately differentiated carcinoma with variation in nuclear shape and structure.



L.W.D.

FIG. 5. Drawings showing blood cell types as seen in bronchial secretion. Wright's stain. $\times 1000$

- A. Blood cell types.
- B. Bronchial epithelial cells.
- C. Variation in cell size, color, nuclear structure from a metastatic carcinoma.
- D. From an anaplastic carcinoma. Note relatively large nuclei, nuclear clefts, nucleoli.
- E. Mitotic figure; attached cytoplasmic material. From patient with proved lung malignancy.
- F. Large cell with nucleolus. There is a questionable nuclear fragment in cytoplasm. From patient with proved lung malignancy.



L. W. D.

FIG. 6. Drawings of cells in bronchial secretion in patients with proved malignancy of lung. Wright's stain. $\times 1000$

A. Group of malignant cells showing variation in nuclear structure with bizarre nuclear shape.

B. Large cell with double nucleus. Basic material is attached to cytoplasm.

C. and D. From a squamous cell carcinoma.

E. Giant cell with multiple nuclei and bizarre unidentified structure.

F. From an undifferentiated carcinoma. Note variation in nuclear structure.

simple and involves minimum equipment and expense. Bacteria, fungi and spirochetes are usually well stained by Wright's method.

The reason for fixing part of the smears, while still wet, in alcohol and ether and for allowing part of the smears to dry in the air is that specimens vary in cellular content and in consistency and often the air-dried specimens reveal less distortion of cellular structures, less interfering precipitated material and better definition of cell details than the fixed specimens.

The essential feature is not the stain used or the method of fixation or sectioning, but rather the proper interpretation by the pathologist who examines the preparation. No staining technics so far developed can replace the skill and judgment in interpretation required of the pathologist.

The staining of smears of aspirated material should not replace the biopsy or other standard technics but should be a supporting and additional procedure. In patients from whom it is impossible to obtain material for biopsy, the examination of aspirated material is most valuable.

The diagnosis of malignancy cannot and should not be made on single cells, but on the finding of many cells which collectively reveal the picture of abnormal morphology of the type associated with malignancy. In case of doubt, the finding observed should be described and the smear reported as suspicious but not diagnostic.

SUMMARY

The ordinary Wright's stain is satisfactory for staining malignant cells in bronchial secretions. The morphology of normal and malignant cells from bronchial aspirations is described and illustrated.

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TRISODIUM PHOSPHATE IN THE DIAGNOSTIC CULTURE OF TUBERCLE BACILLI*

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Three decades of persistent experiment and trial have firmly established the practical value of the cultural method for disclosing the presence of small numbers of mammalian tubercle bacilli in pathologic materials. It now supersedes, in most respects, the more costly animal test which challenged the cultural method because of the high susceptibility of the guinea pig to small numbers of virulent human and bovine tubercle bacilli. The cultural method, however, proved superior in disclosing not only small numbers of virulent mammalian bacilli but also tubercle bacilli of low or moderate virulence and in being free from many uncertainties of the animal test. The most difficult problem to overcome, which the animal method surmounted easily, was the elimination of undesirable contaminating organisms without injury to the virulent tubercle bacilli.

The early use of hypochlorites and, later, of the quickly acting caustic alkalis, despite their tissue-softening and solvent properties, left much to be desired in obtaining successful cultures in the presence of sparse numbers of bacilli. The desire for speed and for pure reagents led, in 1930,⁴ to the successful use of certain acids and to the recommendation of a pure crystalline simple acid, oxalic acid. The disadvantage of the acid, however, was that in spite of its suitable sterilizing effect on contaminants when properly used, it did not possess the liquefying or solvent properties of the strong alkalis, which, therefore, limited its usefulness when thick bulky specimens, such as sputum, were tested. Sodium hydroxide proved more satisfactory in this respect, but still possessed a number of undesirable features. The latter were obviated by the use of another weaker alkali, trisodium phosphate. Trisodium phosphate,⁸ which is also a tissue softener and penetrant, can be obtained in stable, pure crystalline form and can be maintained without deterioration for indefinite periods of time in suitable solution for use in destroying contaminants in tuberculous materials. In a previous communication³ it was pointed out that in 10 per cent solution (23 per cent $\text{Na}_3\text{PO}_4 \cdot 12 \text{H}_2\text{O}$), trisodium phosphate can remain in contact with tubercle bacilli for up to one week at room temperature without destroying small numbers of tubercle bacilli, but it destroys the undesirable contaminants in sputum within twenty-four hours at 37 C., or within a period varying from several days to one week at room temperature. It may also be placed in the receptacles used for collecting the specimen; this immediately prevents the development of molds and undesired contaminants. In ordinary specimens, such as sputum, urine, gastric washings and pus, the time required for destroying contaminants by

* Presented at the Twenty-Sixth Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 29, 1947. Received for publication, October 18, 1947. This investigation was aided by a gift from Morton May in memory of Florence G. May.

trisodium phosphate is one day at 37 C. This time interval adds to the convenience with which the technician can prepare the specimens for culture, since there is no necessity for the haste required with sodium hydroxide or oxalic acid. Van Vranken⁹ used this reagent in combination with the egg yolk medium^{1, 2} in extensive comparative tests and found it to be a superior method for use by a department of public health.

Although this contribution is concerned mainly with the culture of feces, several experiments with sputum are recorded here to elucidate the problems of neutralization and of depositing sputum directly into the receptacle containing trisodium phosphate as a preservative against contaminants. Table 1 records

TABLE 1
EFFECT OF VARIOUS CONCENTRATIONS OF TRISODIUM PHOSPHATE ON GROWTH OF
TUBERCLE BACILLI FROM PERSISTENTLY POSITIVE SPUTUMS

MATERIAL AND TREATMENT						NUMBER OF WEEKS REQUIRED FOR GROWTH				CONTAMINATIONS IN TOTAL OF 12 TUBES PLANTED				
Proportion [†]		B.T.B.† Drops	Incuba- tion at 23 C.	Material Added	Incuba- tion at 37 C.	Sputum Number				Sputum Number				Total
Phosphate*	Sputum					1	2	3	4	1	2	3	4	
			hr.		hr.									
1	1	3			24	4	3	3	4	1	1	1	1	4
1	1	2	24		24	3	3	3	4	1	0	1	0	2
2	1	3	24	3 cc. saline	24	3	3	3	6	0	1	0	1	2
(6 cc.)	(3 cc.)													
4	1	15	24	9 cc. saline	24	3	3	3	3	0	0	0	1	1
(12 cc.)	(3 cc.)													
1	2	9	24	3 cc. Na ₂ PO ₄	24	3	3	3	4	1	2	1	2	6
(3 cc.)	(6 cc.)													
1	4	15	24	9 cc. Na ₃ PO ₄	24	0	3	3	4	3	2	1	1	7
(3 cc.)	(12 cc.)													

* 10 per cent (or 23 per cent hydrated) trisodium phosphate. All mixtures were neutralized with 5 per cent HCl after completing incubation, and the centrifugated, saline diluted, sediment was planted on the medium.

† B.T.B., bromthymol blue, prepared according to Clark: The Determination of Hydrogen Ions, 1920, page 66, The Williams and Wilkins Company, Baltimore.

the results of various concentrations of trisodium phosphate containing bromthymol blue solution (Clark and Lubs) on sputum, the phosphate being in contact at room temperature for one day and then at incubator temperature (37 C.) for one day.

The results recorded in Table 1 indicate that, as far as positive cultures from tuberculous sputums are concerned, there is a wide latitude in the proportion of trisodium phosphate to sputum, varying from 4 to 1 to 1 to 4. However, as the concentration of trisodium phosphate fell below 50 per cent of a 10 per cent solution, the number of contaminations with these sputums increased slightly. Sputums which were positive for long periods of time were chosen for this test since they were more likely to have contaminants.

In a second experiment with 14 sputums, recorded in Table 2, the sputum was collected directly into 10 per cent trisodium phosphate solution containing bromthymol blue indicator. The object was to determine whether a given concentration of trisodium phosphate was detrimental to the recovery of the bacilli and whether dilution and centrifugation influenced this recovery.

It would appear from the results recorded in Table 2 that the amount of 10 per cent trisodium phosphate solution placed in the receptacle in which sputum is collected significantly retards contaminants but does not influence the number of positive cultures of tubercle bacilli. It would appear from these observations that centrifugation should be obviated when possible, since it results in additional contamination and does not concentrate the bacilli advantageously.

The cultural method is highly efficient and is widely used with most specimens. This is not true in the culture of feces which should be a veritable reservoir for tubercle bacilli in tuberculous patients, especially those with pulmonary disease who swallow positive sputum. The use of gastric washings, although requiring care to preserve the viability of the bacilli, has far exceeded the use of feces in popularity. Some early authors^{5, 6} have reported favorably on recovery by culture of viable tubercle bacilli from the feces of the tuberculous, while those who have failed⁷ have offered no valid explanation for their failures. In view of the fact that the latitude in the use of trisodium phosphate was far greater than that of previous reagents used, its application to the isolation of tubercle bacilli from feces appeared worthy of consideration. The following experimental study of a large number of specimens of feces was performed with the purpose of observing whether the isolation of bacilli from the feces might prove of some prognostic or diagnostic value, or, if the bacilli could not be cultured satisfactorily from this source, the reason for the difficulty.

In earlier work it was pointed out that sodium hydroxide, oxalic acid and trisodium phosphate were all incapable, under ordinary conditions, of destroying all the undesirable microorganisms present in feces. The sodium hydroxide or oxalic acid alone proved completely inefficient within the time limit allowed for maintaining viable tubercle bacilli, while the trisodium phosphate alone required from three to seven days at 37 C. to remove these undesirable organisms, a period of time which still did not affect the viability of the tubercle bacilli. In a previous report,³ it was pointed out that certain fungi were destroyed by alkalies only and others by acids only, in a length of time which did not produce injury to the tubercle bacilli. For this reason, in the following studies, the feces were treated with a combination of oxalic acid and trisodium phosphate.

The results in sputum-positive patients with tuberculosis, using both the trisodium phosphate and oxalic acid treatment, were not encouraging and are given here only briefly. Positive cultures of feces were obtained in only 4 of 63 tuberculous women, and in 1 of 55 tuberculous men. Oddly enough, in one of these patients, a positive culture was obtained from the feces on each of four examinations. There appeared to be no correlation between a positive culture from the feces and the clinical stage of the pulmonary disease, whether II or III, moderately or far advanced. One woman was clinically regarded as being spu-

TABLE 2
EFFECT OF DEPOSITING SPUTUM DIRECTLY INTO TRISODIUM PHOSPHATE

TREATMENT OF SPUTUM	NUMBER OF WEEKS FOR GROWTH OF TUBERCLE BACILLI AND NUMBER OF CONTAMINATIONS AMONG 3 CULTURES OF EACH SPUTUM														TOTAL CONTAMINATIONS OF 42 TUBES PLANTED
	Sputum Number														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
A. 5 cc. sputum + 15 cc. 10 per cent Na_2PO_4 ; 20 drops B.T.B.* added over two days; then incubated at 37 C. for twenty-four hours; neutralized with 5 per cent HCl and planted	3 wk.	3 wk.	4 wk.	4 wk.	3 wk.	6 wk.	3 wk.	5 wk.	8 wk.	3 wk.	3 wk.	5 wk.	3 wk.	0 wk.	6
	0	1	0	0	0	1	1	0	1	0	1	0	1	0	
B. Part of A was centrifuged and sediment was diluted with equal volume of saline and planted	3 wk.	3 wk.	0 wk.	4 wk.	3 wk.	4 wk.	3 wk.	5 wk.	0 wk.	3 wk.	3 wk.	0 wk.	3 wk.	0 wk.	13
	1	0	3	2	2	0	0	1	1	1	0	2	0	0	
C. 5 cc. sputum + 15 cc. 10 per cent Na_2PO_4 ; 20 drops B.T.B. added over two days; then 10 cc. saline added and incubated at 37 C. for twenty-four hours; neutralized with 5 per cent HCl and planted	3 wk.	4 wk.	4 wk.	4 wk.	3 wk.	5 wk.	3 wk.	5 wk.	5 wk.	4 wk.	3 wk.	6 wk.	3 wk.	0 wk.	5
	0	1	1	0	0	1	0	0	0	1	0	0	1	0	
D. Part of C was centrifuged, sediment diluted with equal volume of saline and planted	3 wk.	4 wk.	4 wk.	4 wk.	3 wk.	4 wk.	3 wk.	4 wk.	0 wk.	0 wk.	3 wk.	7 wk.	3 wk.	0 wk.	17
	0	2	1	2	1	1	2	0	3	1	0	2	0	2	

* B.T.B., bromthymol blue, prepared according to Clark.

TABLE 3

EFFECT OF VARIOUS CONCENTRATIONS OF TRISODIUM PHOSPHATE ON
CONTAMINATING MICROORGANISMS IN FECES OF PATIENTS WITH
PULMONARY TUBERCULOSIS

TREATMENT OF FECES (1 PART SUSPENDED IN 3 PARTS STERILE WATER)*	NUMBER OF CONTAMINATIONS IN 3 TUBES OF EGG YOLK MEDIUM PLANTED																				TOTAL CONTAMINA- TIONS IN 60 TUBES
	Feces Number																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
5 per cent oxalic acid (3 parts to 1 part feces) incubated for one and one-half hours at 37 C.; 3 cc. 10 per cent Na_3PO_4 added to centrifuged sediment; incubated at 37 C. for one day	3	3	3	3	3	1	2	2	2	3	2	0	2	0	1	3	3	1	3	2	42
10 per cent Na_3PO_4 (4:1) incubated for one day at 37 C.; 2 cc. 5 per cent oxalic acid added to sediment and incubated at 37 C. for one and one-half hours	2	3	0	0	2	0	0	0	1	0	0	0	0	0	1	2	0	0	0	0	11
5 per cent oxalic acid (3:1) incubated for one and one-half hours at 37 C.; 3 cc. 10 per cent Na_3PO_4 added to sediment and incubated at 37 C. for three days	1	0	2	3	2	1	3	0	1	2	3	0	1	0	1	3	1	0	1	0	25
10 per cent Na_3PO_4 (4:1) incubated for three days at 37 C.; 2 cc. 5 per cent oxalic acid added to sediment and incubated at 37 C. for one and one-half hours	2	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	4
5 per cent oxalic acid (3:1) incubated for one and one-half hours at 37 C.; 3 cc. 10 per cent Na_3PO_4 added to sediment and incubated at 37 C., for one week	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
10 per cent Na_3PO_4 (4:1) incubated for one week at 37 C.; 2 cc. 5 per cent oxalic acid added to sediment and incubated at 37 C. for one and one-half hours	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	0	3

* Control tests with suspensions of viable tubercle bacilli disclosed that none of these treatments was detrimental in itself to successful cultural recovery of the bacilli from the neutralized final mixtures.

tum-negative. The low percentage of positive cultures made an explanation essential. Were the reagents at fault or were there other natural factors which prevented the development of an efficient practical method for the culture of tubercle bacilli in feces?

The effect of treatment with trisodium phosphate and oxalic acid on the contaminating microorganisms in feces is shown in Table 3.

The results indicate that in human feces treated with trisodium phosphate

TABLE 4
AN ATTEMPT TO RECOVER HUMAN TUBERCLE BACILLI ADDED TO FECES

TREATMENT OF FECES	NUMBER OF WEEKS REQUIRED FOR GROWTH OF TUBERCLE BACILLI NUMBER OF POSITIVE CULTURES AMONG 3 TUBES PLANTED							
	Feces Number and Cultural Results*							
	63	64	65	66	67	68	69	70
A. 10 per cent Na_3PO_4 (4 parts to 1 part of feces), incubated for one day at 37 C.; neutralized with 5 per cent oxalic acid; 5 per cent oxalic acid added to 0.25 cc. sediment and mixture incubated for one and one-half hours at 37 C.	0	0	0	C.	0	0	0	0
B. 0.001 mg. human tubercle bacilli per cc. of feces suspension; same treatment as A.	0	C.	0	0	0	0	0	0
C. 0.001 mg. human tubercle bacilli per cc. of feces suspension, treated with 10 per cent Na_3PO_4 only for four days at 37 C.	0	C.	0	0	0	4 wk. 1	5 wk. 1	5 wk. 1
D. 0.001 mg. human tubercle bacilli per cc. feces treated with 10 per cent Na_3PO_4 for four days at 37 C.; sediment (0.25 cc.) treated with 1 cc. 5 per cent oxalic acid for one and one-half hours at 37 C.	0	0	0	0	0	0	0	0
E. Same treatment as C but 10 per cent Na_3PO_4 feces mixture incubated one week at 37 C.	0	0	0	0	0	6 wk. 1	0	0

* C, contamination. All mixtures were made neutral to bromthymol blue before planting on the egg yolk medium.

for from three days to one week, and then with oxalic acid for one and one-half hours at 37 C., in most instances, the contaminants present are efficiently destroyed; but in spite of the fact that these reagents do not destroy viable tubercle bacilli in control tests, tubercle bacilli are not recovered from these feces from patients with positive sputums.

In order to test further the recovery of tubercle bacilli, they were added to specimens of feces and the latter were treated with trisodium phosphate and oxalic acid with the results recorded in Table 4. In this experiment, about 1

Gm. of feces was mixed with 5 cc. saline and a fine suspension of tubercle bacilli was added to give a final concentration of 0.001 mg. of bacilli per cc. of mixture. Organisms were recoverable when control suspensions containing the same amount of bacilli were carried through the same treatment of reagent and then incubated for three weeks on the egg medium; organisms were also recovered by culture from suspensions of bacilli not submitted to treatment with reagent after having been stored at 37 C. for more than one week. Duplicate experiments to those recorded in Table 4 with various modifications repeatedly resulted in only occasional recovery of the bacilli in culture.

TABLE 5
SUCCESSFUL RESULTS IN RECOVERY OF ADDED HUMAN TUBERCLE BACILLI FROM
CERTAIN SAMPLE OF FECES BY USE OF TRISODIUM PHOSPHATE

TREATMENT OF FECES	TIME REQUIRED FOR GROWTH OF TUBERCLE BACILLI	
	A*	B†
	<i>weeks</i>	<i>weeks</i>
A. 10 per cent Na_3PO_4 (2 parts to 1 part of feces) incubated for one day at 37 C.; neutralized with 5 per cent oxalic acid; to sediment (0.5 cc.) was added 0.5 cc. oxalic acid; incubated for one and one-half hours at 37 C.	4	2
B. 10 per cent Na_3PO_4 (2 parts to 1 part of feces) incubated three days at 37 C.; neutralized with 5 per cent oxalic acid; 0.5 cc. oxalic acid added to residue (0.5 cc.) incubated for one hour at 37 C.	5	3
C. 10 per cent Na_3PO_4 (2 parts to 1 part of feces) incubated for four days at 37 C.; neutralized with 5 per cent oxalic acid and planted.	3	3
D. 10 per cent Na_3PO_4 (2 parts to 1 part of feces) incubated for one week at 37 C.; neutralized with 5 per cent oxalic acid and planted.	4	2

* A, 1 Gm. feces was well mixed with 0.1 mg. human tubercle bacilli and diluted to 4 cc.; 1 cc. was used for each test.

† B, control suspension of human tubercle bacilli submitted to treatment as indicated.

The findings recorded in Table 4 indicate that some substance in the feces, or possibly some substance liberated by treatment with trisodium phosphate and/or oxalic acid, prevented the recovery of viable tubercle bacilli from the mixtures of feces. The inconstancy of these findings was well illustrated by a sample of feces from which recovery of tubercle bacilli was possible, regularly, after addition of the bacilli, as illustrated in Table 5.

The results recorded in Table 5 are convincing proof that the treatment with trisodium phosphate and oxalic acid does not account for the inability to obtain positive cultures from feces to which tubercle bacilli have been added, but rather

are suggestive that the action lies in the composition of the feces itself, either in containing a material which is toxic to the bacilli, or in the formation of such toxic material by the reagents used.

In order to obtain further information on the direct toxicity of feces to tubercle bacilli or the effect of treatment by the reagent, feces from 8 different patients were autoclaved for thirty minutes at about 118 C. (18 pounds pressure). This treatment was not capable of destroying contaminants completely in some of the specimens of feces. The results of direct planting of material from these fecal specimens on egg yolk medium after mixing intimately 0.001 mg. of suspen-

TABLE 6
RECOVERY OF HUMAN TUBERCLE BACILLI ADDED TO AUTOCLAVED FECES AFTER
SUBMITTING AUTOCLAVED FECES TO VARIOUS TREATMENTS

TREATMENT OF SPECIMEN	NUMBER WEEKS REQUIRED FOR GROWTH TUBERCLE BACILLI*							
	Feces Number							
	118	119	120	121	123	124	125	126
A. 0.001 mg. human tubercle bacilli in feces, planted immediately.	3	5	C.	0	0	0	0	3
B. 0.001 mg. human tubercle bacilli in feces, incubated at 37 C. for three days.	3	0	C.	C.	0	C.	0	C.
C. 0.001 mg. human tubercle bacilli in feces, incubated for one week at 37 C.	C.	0	C.	0	0	C.	0	0
D. 0.001 mg. human tubercle bacilli in feces treated with 2 volumes 10 per cent Na_3PO_4 ; incubated for three days at 37 C.; neutralized before planting.	5	0	0	5	0	0	0	8
E. 0.001 mg. human tubercle bacilli in feces, incubated for three days at 37 C.; 2 volumes 10 per cent Na_3PO_4 added, then incubated at 37 C. for four days; neutralized before planting.	0	0	0	0	0	0	0	0

* C. indicates that all three tubes were contaminated; 0, no growth.

sion of human tubercle bacilli per cc. of mixture of feces and submitting these to various intervals of incubation and treatment with reagent are given in Table 6.

It is noted from the findings recorded in Table 6 that upon immediate planting of the sterilized feces mixed with tubercle bacilli, recovery occurred in 3 of 8 specimens tested; after incubation for three days at 37 C., recovery was obtained from only one specimen of feces. These findings are partly accounted for by contaminations which were eliminated in the reagent-treated specimens where more recoveries occurred. After seven days at 37 C., however, neither the specimens planted directly, nor those treated with reagent yielded positive cultures for tubercle bacilli. It would appear from this that the toxic material in

the feces is relatively weak, but that it is definitely active in destroying the viability of the tubercle bacilli, even when the latter are added to autoclaved feces.

In another experiment in which the fecal specimen was submitted to two short autoclavings on succeeding days, in order to eliminate the contaminants more efficiently, the toxic effect of the feces still persisted. This is indicated in the results recorded in Table 7 and is evident after three days' incubation at 37 C. of the mixture of feces and tubercle bacilli in spite of the addition of the trisodium phosphate reagent.

Attempts to obtain a soluble filterable product from feces which would account for this toxicity to tubercle bacilli met with failure. Seitz filtrates from feces kept at incubator temperatures or for days at refrigerator temperature, as well as watery extracts from autoclaved feces, did not destroy tubercle bacilli within

TABLE 7

RECOVERY OF HUMAN TUBERCLE BACILLI ADDED TO AUTOCLAVED FECES FOLLOWING TREATMENT OF AUTOCLAVED FECES WITH TRISODIUM PHOSPHATE

TREATMENT OF SPECIMEN	NUMBER OF WEEKS REQUIRED FOR GROWTH*							
	Feces Number							
	136	137	138	139	140	141	142	143
A. 0.01 mg. human tubercle bacilli per cc. feces† and 10 per cent Na_3PO_4 (2:1 feces) added, neutralized immediately and planted.	3	3	3	3	3	3	3	3
B. 0.01 mg. human tubercle bacilli in 1 cc. of feces, 10 per cent Na_3PO_4 (2:1 feces) added, kept for three days at 37 C., neutralized and planted.	0	0	0	0	0	0	0	0
C. 0.01 mg. human tubercle bacilli in 1 cc. feces, 10 per cent Na_3PO_4 (2:1 feces) added, kept for one week at 37 C., neutralized and planted.	0	0	0	0	0	0	0	0

* 0 indicates no growth.

† Suspensions of tubercle bacilli containing 0.01 mg. per cc. and kept at 37 C. for one week are readily recoverable regardless of whether they are planted directly or treated with trisodium phosphate as above.

a reasonable time. Trisodium phosphate extracts of feces after incubation for three days at 37 C. did not kill them, nor did hot ether and chloroform extracts of feces. Attempts to pass avirulent human tubercle bacilli given by mouth through the gastro-intestinal tracts of dogs and rabbits on a standard diet were unsuccessful because of the heavy contamination of these feces which could not be prevented by either sodium hydroxide, oxalic acid, or trisodium phosphate, when used alone, or in combination, or consecutively in amounts and in times of exposure which did not, of themselves, affect the viability of tubercle bacilli.

Thus, the obvious conclusions to be drawn from these studies are that tubercle bacilli placed in feces slowly lose their viability as a result of substances present in the fecal material itself and that the speed with which mammalian tubercle bacilli lose their viability in feces varies according to the composition and nature

of the individual fecal specimen. The loss of viability is usually complete in several days at 37 C. and probably also occurs to a great extent in the passage through the gastro-intestinal tract.

SUMMARY AND CONCLUSIONS

Trisodium phosphate, previously used for destroying undesired contaminating microorganisms in sputum, urine, gastric washings and purulent materials from human or animal sources for the culture of mammalian tubercle bacilli, has proved valuable in that it acts over a long period of time as compared with previously recommended reagents. It acts efficiently at 37 C., and can be kept in contact with tuberculous materials for up to one week at room temperature (20 to 25 C.) without detriment to the contained tubercle bacilli.

Neither the new trisodium phosphate reagent, nor the sodium hydroxide, nor oxalic acid alone or in combination, proved satisfactory for the isolation of mammalian tubercle bacilli from feces even though prolonged or combined action succeeded in eliminating contaminating microorganisms from most feces. In most instances, the feces destroyed the viability of naturally-contained or added viable tubercle bacilli within several days, when incubated at 37 C. This may account for the fact that previous attempts to use this source of human or animal material for diagnostic purposes have not met with success. An occasional fecal specimen from a tuberculous patient does not have this detrimental action on tubercle bacilli, since from such a specimen, the tubercle bacilli may be recovered regularly by the use of the trisodium phosphate reagent alone, or following treatment by oxalic acid.

The nature of the substances in the feces responsible for destroying the viability of the tubercle bacilli was not disclosed.

Trisodium phosphate is a valuable reagent for destroying undesirable contaminants in pathologic specimens for the cultural diagnosis of tuberculosis.

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THE PATHOLOGY OF HYPERSPLENISM*

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Pathologic hyperfunction of the spleen has been considered as the basis for a number of blood dyscrasias quite different in their clinical manifestations. Two of them, hemolytic jaundice and thrombocytopenic purpura, have been recognized for some time, while two others, splenic neutropenia (Wiseman¹²) and splenic panhematocytopenia (Doan²) have more recently been described. All four conditions are often greatly benefited by splenectomy, which frequently has become recognized as a life-saving measure in the clinical management of these blood dyscrasias. According to Doan, the diagnosis of a splenic blood dyscrasia rests mainly on five important criteria: diminution of one or more of the circulating elements of the blood, normal or increased bone marrow activity, splenic enlargement, increase in the number of deficient blood elements after subcutaneous injection of adrenalin and complete rapid clinical recovery of the patient following splenectomy.

The pathologic picture of the overactive spleen has been exhaustively studied in various disease conditions. Klemperer,⁷ in his monograph, gave an excellent classification of the so-called "*splenic tumor*" and described its various histopathologic types. Eppinger,³ Freund,⁴ Thompson¹⁰ and others studied the histology of the spleen in hemolytic jaundice and emphasized the marked congestion of the pulp in contrast to the empty sinusoids. Some authors reported hyperplasia of the reticulum of the red pulp, while others claimed that the histologic evidence of increased blood destruction was not constant and that erythrophagocytosis and siderosis were conspicuous in some cases and absent in others.

Eppinger, favoring the theory of the splenic etiology of *hemolytic icterus*, stressed the characteristic engorgement of the red pulp. He maintained that this engorgement was dependent on circulatory alterations, and described some abnormalities of splenic arterioles which he held responsible for that phenomenon. Klemperer also believed that circulatory alterations were responsible for the congestion, but attributed them to abnormalities of the red blood cells and not of the arteries, and he failed to find the arterial lesions described by Eppinger.

The histology of the spleen in *thrombocytopenic purpura* also has been variously described by different authors. Hinschberger *et al.*,⁵ Weil and Grégoire,¹¹ Levrat,⁸ MacCarty,⁹ Bergqvist¹ and Klemperer⁷ reported that the histologic picture did not present either characteristic alterations or lesions which could account for the pathogenesis of the disease. Kellert⁶ reported a histologic similarity between essential thrombocytopenia and infectious enlargement of the spleen. Whitby and Britton¹³ described an endothelial proliferation of the

* Presented at the Twenty-Sixth Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 29, 1947. Received for publication, October 29, 1947.

malpighian follicles and sinuses with an increase in the number of the reticulum cells throughout the whole organ, with considerable infiltration of the pulp with polymorphonuclear leukocytes and eosinophils. Megakaryocytes are sometimes found, while platelets may be extensively phagocytosed. With regard to the phagocytosis of platelets, Klemperer stated that the great cellularity of the spleen renders a recognition of platelets exceedingly difficult in tissue sections. Only once was he able to recognize phagocytosed platelets inside the macrophages in the spleen among several cases of thrombocytopenic purpura examined by him. No reference to the histologic picture of splenic neutropenia or hematopenia is available except the supravital studies done by Doan and Wiseman.

The controversy of the various reports on the histopathology of the spleen in splenic blood dyscrasias and the lack of histologic studies of the syndrome of splenic neutropenia and panhematopenia initiated our study of the pathology of hypersplenism.

MATERIAL

The spleens used in our investigations were removed at operation or autopsy. The spleens, the weights of which were recorded immediately after removal, were in part fixed in various histologic fixatives and in part ground up for biochemical studies. Smears for supravital studies were obtained from the freshly removed organ and stained with the usual technic.

A total of 184 spleens, 134 of which were removed at operation, were studied histologically. The following clinical grouping was chosen:

1. Hemolytic icterus	45 cases
2. Thrombocytopenic purpura	36 cases
3. Splenic neutropenia	11 cases
4. Splenic panhemocytopenia	10 cases
5. Secondary hypersplenism	32 cases
6. Control spleens	50 cases

The group of secondary hypersplenism included 3 cases each of Gaucher's disease and Hodgkin's disease, 2 cases each of chronic lymphatic leukemia, Felty's syndrome and hemangioma, 1 case each of myelogenous leukemia, Boeck's sarcoid, moniliasis and tuberculosis, and 16 cases of Banti's syndrome.

The control group comprised spleens of healthy individuals who died in accidents (27 cases) and spleens removed from patients dying from various infectious, degenerative and neoplastic diseases (23 cases). The ages ranged from 3 months to 84 years, and the patients included both sexes and white and Negro races.

METHODS

I. Histologic Investigation

The following methods were adopted for a histologic evaluation of any lesions present. Several sections from each patient were chosen so that capsular and hilar regions of the organ could be studied. The following differential stains were used: the hematoxylin-eosin as used routinely in our laboratory; the

trichrome stain modified by Foot; Wilder's stain for reticulum fibers and Weigert's elastic tissue stain. For systematic studies of sections the following sequences of observations was adhered to:

1. The capsule and trabecular system, including the vascular tree, were studied. Special attention was given to the width and distribution of trabeculae and thickness of capsule. The varying amounts of smooth muscle, elastic and reticulum fibers in these structures were noted and intertrabecular hemorrhages and infiltrations were recorded. Of the vascular tree, sections of trabecular arteries and veins, follicular arteries and sheathed arterioles were studied with regard to thickness of the wall and character of endothelial lining, smooth muscle fibers and elastic fibers.
2. Malpighian corpuscles present in the organ were studied, giving special attention to size and distribution, presence or absence of germinal centers, width of the lymphocytic zone and presence or absence of a peripheral endothelial zone.
3. The sinusoids of the spleen were investigated with regard to their state of dilatation; their endothelial lining and their content.
4. The splenic pulp was studied from the point of view of its relative amount, its content of reticulum cells, reticulum fibers and blood cell elements and the various processes of phagocytosis observed in the pulp.

By listing all these observations objectively in tabular form, an attempt was made to find those changes which reoccurred most characteristically and to differentiate them from those which may be regarded as incidental findings. A discussion of these findings as elicited for each group will be given below.

II. Cytologic Methods

Our cytologic studies consisted of cell counts on fixed tissue sections stained with hematoxylin-eosin and qualitative cell studies of freshly obtained splenic imprints stained with Giemsa's and supravital stains. In our cell counts the reticulum and endothelial cells as recognized by the nuclear characteristics in the hematoxylin-eosin stain were counted regardless of their location or phagocytic activity. One hundred square fields were counted in each section with an oil immersion lens and a total magnification of 930 x. The counts were compared with those found in control spleens, and from it and the weight difference the probable increase of splenic cells was estimated. While we realized that this method was crude, it was used to confirm or reject the assumption of "tremendous increase of phagocytic power" as emphasized by some authors.

Our qualitative studies on splenic imprints were mainly directed to the study of the process of phagocytosis without any attempt at quantitative estimation. An attempt was made to differentiate the type of blood dyscrasia by the type of phagocytic activity demonstrated in these splenic imprints.

RESULTS

I. Histologic Findings

Group 1 consisted of 42 patients with congenital and 3 patients with *acquired hemolytic icterus*. All the patients in the congenital group gave a definite family history. The patients' ages varied from 8 to 74 years. The outstanding clinical and hematologic findings were anemia, jaundice, splenic enlargement, high

reticulocyte count, increased erythrocyte fragility and a bone marrow which showed hyperplasia of the erythroid elements with a shift towards less mature forms. The histopathology of the spleen in these cases showed a marked increase in the amount of red pulp. This increase was due to extreme engorgement with red blood corpuscles and hyperplasia of the reticulo-endothelial cells of the red pulp. Evidence of erythrophagocytosis was present in some patients and absent in others. Iron stains were made in all cases and hemosiderosis was conspicuous in 35 instances. The red pulp contained few neutrophilic leukocytes and fewer eosinophils. The sinusoids were almost always compressed by the congested red pulp, and only a few, partly dilated sinusoids could be found. These contained red blood cells, leukocytes and phagocytic cells containing red blood cells in various stages of disintegration. The endothelial cells lining the sinusoids, as well as the endothelial cells of the ellipsoids, showed in all patients definite phagocytic activity with or without hemosiderosis. The lymphoid follicles of the spleens in hemolytic icterus did not show any abnormality. The perifollicular wall of reticulo-endothelial cells was prominent in all patients regardless of age. The follicles showed the usual variation in size and distribution. Large follicles with prominent germinal centers were observed in the younger patients of this group. The capsule of the spleen did not show any specific lesion. Evidence of splenic enlargement was observed in the form of widening of the intertrabecular spaces. The arteries and arterioles appeared normal.

The second group consisted of 36 patients with *essential thrombocytopenic purpura*. The ages varied from 8 months to 64 years. All cases showed a low platelet count before splenectomy. The spleen was clinically enlarged in 19 instances, and the splenic weights varied from 56 to 248 Gm. The essential pathologic findings in the pulp were a slight to moderate increase in the width of the Billroth's cords, which were conspicuous for their remarkable cellularity. Most of the cells filling the meshes of the pulp belonged to the reticulo-endothelial group. There was little evidence of phagocytosis. There were also numerous polymorphonuclear leukocytes and a few eosinophils. Nodular accumulations of cells were observed frequently which resembled Malpighian follicles except for the absence of lymphocytes and germinal centers. They consisted purely of reticulo-endothelial cells and infiltrated with finger-like protrusions into the surrounding pulp. Sometimes these "pseudofollicles" attained a larger size than the actual lymphoid follicles in the same section. Platelet-like material could be noticed in uneven distribution in the dilated sinuses, but no definite evidence of platelet phagocytosis was observed. The splenic sinusoids were usually dilated. Some sections showed marked perifollicular engorgement of the sinusoids with red blood corpuscles. Other sinusoids were either empty or contained a few mixed blood cell elements. All but 6 cases showed clumped minute particles very suggestive of platelet thrombi. They were found either obstructing the lumens of sinusoids or along the endothelial borders. The lymphoid follicles were enlarged in all patients. The germinal centers were prominent in all patients under the age of 40. In most of the subjects, besides the usual primitive endothelial cells, there were large pale-staining cells showing a vacuolated cyto-

plasm and large vesicular nuclei with prominent nucleoli. An outstanding finding was the presence of a perifollicular zone of reticulum cells irrespective of the age of the patient and the size of the spleen.

The third group included 11 patients with *splenic neutropenia*. The principal clinical and laboratory findings were enlargement of the spleen with peripheral granulocytopenia and a pronounced hyperplasia of all myeloid elements of the bone marrow. Histologically, the principal findings consisted of the enlargement of the Billroth's cords, which was due to hyperplasia of the reticulo-endothelial elements. Many of these cells showed definite evidence of phagocytosis of granulocytes recognizable as nuclear fragments in the cytoplasm of those cells. Erythrophagocytosis was only noticed in Cases 1, 4 and 6, and the last case showed marked hemosiderosis. As a rule the pulp was rather devoid of red blood cells but it contained a definitely increased number of polymorphonuclear leukocytes. The sinusoids of the spleen were dilated in all instances. They contained a conspicuous number of white blood cells and in 5 patients, macrophages with engorged granulocytic material were observed. The endothelial cells lining the sinusoids showed an increased phagocytic activity towards red blood cells but not towards white blood cells. In all but two patients (Cases 1 and 4), the lymphoid follicles were enlarged. They were widely separated and unevenly distributed. The germinal centers were prominent and the perifollicular zone of reticulum cells was quite distinct in all cases regardless of the age of the patient. Case 4 showed a similar nodular accumulation of reticulum cells as described in the preceding paragraph, which resembled Malpighian follicles but lacked lymphocytes and germinal centers.

Group 4 included 10 cases of *panhematocytopenia*. Any patient showing a marked peripheral depression of more than one type of formed elements in the blood was included in this group. Many showed a definite depression of all formed elements. The ages of the patients varied from 2 to 71 years. Splenic enlargement was present in all patients, and the bone marrow showed a diffuse generalized hyperplasia. Again, the histologic findings in the spleen consisted of a moderate to marked increase in the size of the splenic pulp due to hyperplasia of reticulo-endothelial cells and infiltration of the pulp with various formed blood elements. Erythrophagocytosis and granulophagocytosis could be observed in various stages. The sinusoids of the spleen were rather dilated and contained numerous white blood cells and a few red blood cells. There were also numerous macrophages present in the sinusoids. The vascular endothelium displayed distinct erythrophagocytosis in Cases 2, 5, 6, 7, 9 and 10, and the degree of hemosiderosis was increased in all cases. The accumulation of platelet-like elements could be observed, although it was never prominent. The lymphoid follicles were particularly enlarged in Cases 1, 5 and 7. Two cases showed definite hyperplasia of the germinal centers with vacuolated cells containing a large vesicular nucleus. All cases showed a well developed perifollicular zone of reticulum cells. In Cases 2, 5, 6, 7, 9 and 10 this perifollicular zone showed intense congestion.

Group 5 included 32 cases of *various clinical disorders* which had as a common

clinical finding enlargement of the spleen and depression of one or more of the formed elements in the peripheral blood. This depression was assumed to be due to secondary hypersplenism, as the bone marrow showed evidence of compensatory hyperplasia of these elements. Splenectomy was curative with regard to the specific blood cell deficiency without having any influence on the course of the underlying disease.

Histologic examination of the spleen showed varying pictures according to the disease present in the spleen. Thus, the patients with Gaucher's disease showed an enormous hyperplasia of the pulp with the typical lipid-storing cells characteristic of the disease. Erythrophagocytosis and, rarely, phagocytosis of granulocytes were observed, particularly in Case 1. The 3 cases of Hodgkin's disease showed invasion of the splenic pulp by typical granulomatous tissue with fibrosis and numerous Paltauf-Sternberg giant cells. Evidence of erythrophagocytosis was present in two cases. The 16 cases of Banti's syndrome showed uniformly the picture of fibro-adenia as typical for the spleen in this condition. The cellularity of the Billroth's cords varied greatly in the individual cases, however, as did the sequestration of formed blood elements in the dilated sinuses. It was this cellularity of the red pulp which distinguished most of the spleens in this group from the spleen of Laennec's cirrhosis, which shows only fibrosis of the pulp without much reticulum cell proliferation. Particularly outstanding was the leukocyte sequestration in the 2 cases of Felty's syndrome, which otherwise also showed the picture of splenic fibro-adenia. The subjects with leukemia showed rather circumscribed hemolytic foci in the leukemic pulp. These foci were characterized by an area of dilated and engorged sinusoids with reticulum-cell proliferation in the red pulp. These foci were relatively free of leukemic cells and appeared like islands in the mass of leukemic infiltration. The three inflammatory lesions of the spleen (Boeck's sarcoid, moniliasis and tuberculosis) did not show any conspicuous pathologic changes other than those indicated by the diagnosis. All three infections, however, are definitely known to stimulate proliferation of the reticulo-endothelial cells, and it is this stimulation which may have been responsible for the clinical picture of splenic hyperactivity. Of the 2 cases of hemangioma, 1 was cavernous, and 1 was diffuse. In both cases a slight depression of the peripheral blood elements was present which was relieved by splenectomy. The histopathologic picture of the spleen showed the tumors in an otherwise normal organ.

Group 6 consisted of 50 spleens which served as *controls*. In 27 cases, death resulted from accidents and there was no splenic lesion. In 23 cases, death was attributed to various infectious or neoplastic conditions. The ages of the patients varied from 1 month to 84 years. Since these patients were not suspected of having any blood dyscrasia, no study other than the routine peripheral blood count was available. The purpose of including this group in our series was to compare the histologic picture present with that found in the conditions of clinical hypersplenism. It was felt that such a comparative study was absolutely necessary in order to identify and properly evaluate any pathologic changes supposedly characteristic of functional splenic hyperplasia. In 15 cases a slight hyperplasia

of the reticulo-endothelial elements was present. A few of the cases showed moderate hemosiderosis. Erythrophagocytosis was absent. A comparison of the congestion of the pulp in instances of hemolytic jaundice and in control spleens led to the observation that congestion of the pulp in the control spleens was always secondary to the more marked congestion of the sinusoids. In contrast to this the sinusoids of the spleens in hemolytic jaundice were conspicuously empty and compressed. In the cases of acute infectious "splenic tumors" with infiltration of the pulp by polymorphonuclear leukocytes, the similarity to the histologic picture found in splenic neutropenia cannot be denied. However, neither the diffuse nor the perifollicular reticulum cell hyperplasia was present. In 26 cases, the sinusoids of the spleens were collapsed, while in 16 other cases, the sinusoids were patent. The collapse of the sinusoids was not accompanied by an increase in the size of the pulp. It represented merely a sign of contraction and atrophy of the organ. A varying degree of hemosiderosis was found in the histologically normal spleens, but erythrophagocytosis was universally absent in the sinusoids. The size of the lymphoid follicles had a definite relationship to the age of the patient. Large follicles with prominent germinal centers and well-defined perifollicular zones of reticulocytes were observed in patients up to 30 years of age, particularly in those who died from accidents. In older individuals, or those suffering from a chronic disease, the follicles were distinctly atrophic.

II. Cytologic Findings

Table 1 indicates the results of our total estimates on the increase of reticulum cells in the various cases of active clinical hypersplenism. The first 8 cases in the table serve as controls, representing individuals, varying in age from 7 to 55 years, who died accidentally. In 13 cases the spleens were removed for various types of clinically active hypersplenism. The normal weight of the adult spleen for our computation was taken as 150 Gm. The normal splenic weights of individuals from 1 to 20 years of life were taken from the table listed in Cowdry's *Problems of Aging*, page 162. The average normal number of reticulo-endothelial cells per 100 oil immersion fields was found to be approximately 6000. From these computed normal standards the increase or decrease in the total number of reticulo-endothelial cells in the organ was roughly estimated and expressed as a factor in the right-hand column of the table. It can be seen that the normal control spleens all fall approximately within the normal standards with the exception of the spleen of a 55 year old patient who died of severe burns, the spleen showing marked atrophy with decrease in cellularity. The cases of thrombocytopenic purpura all showed a total increase in reticulo-endothelial cells although the amount of the increase was found not as impressive as may have been assumed. Neither the splenic weight, nor the cellularity as observed in the fields counted, totaled more than three times the available amount of reticulo-endothelial elements. From this very rough estimate we can state that, at least for the group of thrombocytopenic purpura, no enormous increase of the reticulo-endothelial cells was observed. We do believe that the number of our cases in

other subgroups is too small to be conclusive, but the data would suggest that cases of hemolytic icterus show an impressive increase in reticulo-endothelial elements.

Studies on fixed smears stained by Giemsa's method and on smears stained by the supravital method have led us to no tangible conclusions. While it was possible to observe an increased amount of endothelial cells in these smears, we could not convince ourselves that this increase was overwhelming, or that the

TABLE 1
ESTIMATION OF INCREASE IN RETICULO-ENDOTHELIAL CELLS IN VARIOUS
CONDITIONS OF HYPERSPLENISM

CLINICAL DIAGNOSIS	AGE	WEIGHT OF SPLEEN	RETICULO- ENDOTHELIAL CELLS	FACTOR OF TOTAL INCREASE
		<i>Gm.</i>	<i>per 100 fields</i>	
Carbon monoxide poisoning (e).....	23	280	4910	1.5
Cyanide poisoning (e).....	30	125	7208	1.2
Automobile accident (e).....	7	65	6247	0.8
Contusion of brain (e).....	29	150	5814	1.05
Fracture of skull (e).....	55	100	5638	0.6
Severe burns (e).....	55	50	3940	0.2
Extensive burns (e).....	27	150	6219	1.1
Pulmonary embolism (e).....	20	150	5906	1.0
Thromboeytopenie purpura.....	1	75	9226	3.3
Thromboeytopenie purpura.....	20	180	8318	1.7
Thromboeytopenie purpura.....	10	89	8869	1.7
Thromboeytopenie purpura.....	8	56	9096	1.2
Thromboeytopenie purpura.....	5	94	7843	2.1
Thromboeytopenie purpura.....	20	240	7586	2.0
Splenic neutropenia.....	29	320	9755	3.5
Splenic neutropenia.....	7	650	11,004	15.3
Congenital hemolytic ieterus.....	38	794	11,826	10.4
Congenital hemolytic ieterus.....	15	320	10,912	5.9
Congenital hemolytic ieterus.....	23	2200	9874	24.2
Panhematocytopenia.....	2	140	8598	3.4
Panhematocytopenia.....	34	210	10,316	2.3

(e) = control.

phagocytosis as shown by these stains was markedly increased. We do believe that neither the supravital nor the fixed smear from the spleen can lend itself successfully to differential diagnosis between normal and hyperactive splenic conditions.

DISCUSSION

From our histologic study of spleens in 134 cases of clinical hypersplenism, we must conclude that the reticulo-endothelial cell is the cell involved in this process. The lymphocytic elements seem to play no role in the picture of hypersplenism. In very severe cases of panhematocytopenia the increase of reticulo-endothelial cells throughout the pulp is very marked and diffuse. In milder cases the in-

crease is focal and attention is directed to the formation of numerous "pseudo-follicles", which really represent a nodular form of reticulum cell hyperplasia. In all forms of hypersplenism the reticulum cells in the perifollicular zone seem to increase first. This is not always noted in the cases of secondary hypersplenism, where the reticulocyte proliferation depends largely on the primary splenic disease. The phenomenon of stagnation or sequestration of blood elements in the pulp and the sinusoids is the second outstanding observation in hypersplenism, while that of active phagocytosis must be regarded as less important. Sequestration involves specifically those formed blood elements which are found missing in the peripheral blood stream and it takes place in the meshwork of the red pulp and the sinusoids alike. Small clusters of the deficient blood elements in various degrees of disintegration are found in the distended sinusoids of the spleen and are also scattered diffusely throughout the pulp. We are inclined to ascribe more importance to the accumulation of these blood cell elements in the meshwork of the pulp, since it is at this place that the most intimate contact between these cells and the reticulum cells can be established. Comparison of the clinical and pathologic data showed good agreement between the peripheral blood picture and the splenic histopathology. In most of the cases splenectomy was done as an emergency procedure and, therefore, the histologic picture of the acute phase of hypersplenism was present. In a few cases of hemolytic jaundice splenectomy was performed after the patient had recovered from his acute hemolytic crisis. In those cases histopathologic evidence of blood cell destruction was absent.

A comparison between the histologic and cytologic observations permits an interesting deduction. It seems obvious that the process of blood destruction in the hyperplastic spleen cannot be explained by the process of phagocytosis alone. This is well in accord with our observation that the clinical picture can be invoked by small accessory spleens which obviously do not possess more phagocytic capacity than that of an average enlarged lymph node. All this points to a humoral factor which either actually destroys formed elements of the blood or renders them destructible. Our chemical investigations on some of the material were directed towards the study of such humoral mechanisms. While the results will be published in detail at a later date, we may point out here that we feel that such a humoral factor can be proved and that it exerts a hemolytic and cytolytic effect upon stagnant formed blood cell elements. Such stagnation of formed blood elements in the pulp and sinusoids of the spleen was considered by us to be the second most important finding of clinical hypersplenism.

SUMMARY

1. Histopathologic studies of the spleens removed in 102 cases of essential hypersplenism showed diffuse and nodular hyperplasia of the reticulum cells to a degree which was generally proportional to the severity of the disease.

2. In addition to the reticulum cell hyperplasia, sequestration in the red pulp and splenic sinusoids of the decreased formed elements of the blood could be observed in all those cases in which splenectomy was performed during the acute stages of the hemocytopenia.

3. In 32 cases of secondary hypersplenism small hemolytic or cytolytic foci could be found in the spleen. Most of these patients suffered from some other disease involving the reticulo-endothelial system.

4. Cytologic examinations revealed an increase of the phagocytic power of the enlarged spleen which, however, was not excessive.

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CLINICOPATHOLOGIC CONFERENCE*

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CLINICAL DATA

Presented by Dr. Harold N. Segall, Internist

The patient, a 64 year old white man, a tailor by trade, was admitted to the hospital on October 4, 1947, after two days' illness at home. On October 2, at about 9:00 a.m., while at work, the patient was seized with pain in the chest, which radiated to his arms. This pain lasted for some time but was not particularly severe. He went home for lunch and shortly afterwards the pain recurred. This time the pain was more severe and radiated to both elbows and wrists. It was associated with a sense of pressure under the lower sternum, and with sweating and dyspnea. His physician administered morphine which relieved him in about twenty minutes. On the following day he continued to have severe, knifelike substernal pain and was admitted to the hospital on October 4, on the third day of his illness.

His past clinical history was noncontributory, but the patient stated that he had had "bronchitis" for many years. On June 8, 1947, he had a prostatectomy performed at this hospital, at which time his nonprotein nitrogen was 24.5 mg. and his blood creatinine was 1.17 mg. per 100 ml.

On his present admission to the hospital his temperature was 101 F., the pulse rate 88 per minute, and the respiratory rate 36 per minute. His blood pressure was 98/64 mm. Hg. The patient was well developed and in no apparent acute distress. The heart sounds were regular but distant and no friction rub could be heard over the precordial area. Otherwise, the physical findings were not abnormal.

Electrocardiograms taken on the day of admission showed gross irregularities which were interpreted as "acute myocardial infarction involving the inter-ventricular septum and anterior wall of the left ventricle". On admission urinalysis revealed a specific gravity of 1.020, a trace of albumin and much pus and bacteria. His initial hemoglobin was 91 per cent and his leukocyte count was 6800 per cu. mm.

On the third hospital day, the patient complained of pain in the left upper quadrant, with pain radiating toward the pubis. Deep palpation in the left upper quadrant revealed tenderness and Dr. Messinger, who saw him at this time, suggested the possibility of left renal infarction. The urine, however, on this day failed to show the presence of red blood cells.

On his fifth hospital day, at 5:00 a.m., the patient complained of a sudden pain, numbness and coldness in the left leg below the knee. Examination revealed definite lowering of the skin temperature and pallor of the left foot and

* Received for publication, January 19, 1948.

toes, with poor capillary return. The left femoral and popliteal pulsations were present and equal, but the dorsalis pedis and posterior tibial pulsations were absent. There was diminished sensation to pain and deep pressure in the left leg to the level of the patella. A diagnosis of embolism to the left popliteal artery at the bifurcation was considered. The prothrombin time of the patient was determined and found to be 30 seconds (normal 25-30 seconds), and the patient was given 200 mg. of Dicumarol. The head of the bed was elevated, the left leg was wrapped in cotton and 50 mg. of heparin and 1/2 gr. papavarine were given intravenously.

By 11:00 a.m. the patient was still in pain, and at this time no pulsations could be felt in the left popliteal artery. The patient was seen by Dr. Mark Kaufmann, who ordered spinal anesthesia, and at 1:00 p.m. of that day embolectomy was attempted. Both the femoral and popliteal arteries were opened on the left, but no embolic masses could be found in either situation. The arteries were found to be in spasm, with diminished blood flow in both vessels.

Ischemic necrosis of the left foot progressed. The patient was given intravenous heparin by continuous drip. On October 10 (his seventh hospital day), the prothrombin time had risen to 230 seconds. Heparin was discontinued, and the patient was given large doses of Vitamin K. He vomited some dark brown material, which was attributed to the greatly prolonged prothrombin time. Urinalysis revealed from 3 to 6 red blood cells per high power field and albumin (2 plus). On the next day the prothrombin time was 40 seconds.

On the ninth hospital day, the patient began to hieeough and looked more ill than previously. He appeared to be going into peripheral circulatory collapse and exhibited pallor and clamminess and a drop in blood pressure. His condition, however, improved somewhat by the end of the day.

On the eleventh hospital day it was found that the blood chemistry, which had been taken the previous day, showed a nonprotein nitrogen level of 223 mg., and sugar of 200 mg. per 100 ml. Additional blood was taken to check these figures. On the afternoon of this day the patient began to fibrillate and an electrocardiogram also showed auricular flutter. Amputation of the left lower extremity, which had been scheduled for the following day, was canceled by Dr. Kaufmann because of the evidence of renal damage. Urinalysis now revealed a specific gravity of 1.012, a trace of albumin, numerous pus cells and bacteria, and 3 red blood cells per high power field. The leukocyte count had risen to 18,700 per cu. mm., and the prothrombin time was 28 seconds. The patient gradually became semicomatose and developed pulmonary edema that evening. He was digitalized and given quinidine.

On the twelfth and last hospital day the patient was drowsy. His eyes rolled upward and his chest was full of rhonchi. The blood pressure was 100/60 mm. Hg. The blood findings of the previous day were: nonprotein nitrogen, 235 mg.; creatinine, 8 mg.; total proteins, 5.62 Gm. per 100 ml.; and carbon dioxide combining power, 29.6 volumes per cent. Amputation of the left lower extremity was performed. At operation the femoral artery was found to be thrombosed

and did not bleed. Smaller vessels bled and were ligated. The patient expired about five minutes after the operation was completed.

The clinical diagnoses were: (1) Coronary thrombosis, with infarction of the anterior aspect of the left ventricle and interventricular septum; (2) mural cardiac thrombus, with peripheral emboli to left femoral artery and (?) left kidney; (3) gangrene, left leg, with midthigh amputation; (4) terminal congestive failure; and (5) remote prostatectomy.

CLINICAL DISCUSSION

Dr. H. N. Segall. "On the last day of this patient's life consultation was held with Dr. Mark Kaufmann of the Surgical Department. It was my opinion that the toxic effect of the gangrenous leg had increased the load on the arteriosclerotic kidneys of this patient, and I urged immediate amputation. I believed that the patient would die unless the amputation was performed. Dr. Kaufmann, however, was not enthusiastic about performing an amputation as the man was an extremely bad risk. Upon some insistence, however, he undertook the operation."

Dr. M. Raff. "What was the patient's urinary output?"

Dr. M. Cooperberg. "The urinary output was not recorded, but during the last three or four days the patient was incontinent and accurate estimation was impossible."

Dr. M. Cramer. "Was a splenic infarct considered?"

Dr. Segall. "No, a splenic infarct was not considered. I should like to point out that on the day that Dr. Messinger suspected renal infarction the urine failed to show any red blood cells. While it is true that from 3 to 6 red blood cells per high power field were found in the urine on the 10th and 14th of October, respectively, I must add that we would expect to find many more red blood cells in the urine in renal infarction."

Dr. M. Ratner. "I am not familiar with this case at all, but I have seen renal infarction without any red blood cells in the urine."

Dr. M. A. Simon. "I think that Dr. Ratner's observation is of considerable significance. While it is true that red blood cells in the urine are to be expected in renal infarction, it is apparent that such infarction may occur without red blood cells in the urine. This phenomenon has been observed by the group at the Massachusetts General Hospital,* and I think it is a point of considerable importance to us all.

"I should like to ask Dr. Kaufmann if he thought there was absence of pulsation in both the left femoral and popliteal arteries at the time of embolectomy."

Dr. M. Kaufmann. "The femoral pulsation was weak but could be obtained. However, no pulsations in the popliteal and dorsalis pedis arteries could be felt. I opened the femoral artery, but I did not get very active blood flow, in spite of

* Case records of the Massachusetts General Hospital, Case 33311, *New England J. Med.*, 237: 163-167, 1947; and Case 33421, *New England J. Med.*, 237: 590-593, 1947.

going quite high. I then opened the popliteal artery and found the vessel in spasm. In neither situation did I discover an embolus."

DISCUSSION OF PATHOLOGIC FINDINGS

Dr. Simon. "At autopsy, the pericardial sac was distended and contained a large quantity of blood. The exact amount of blood could not be determined



FIG. 1. Photograph of heart showing adherent organizing blood clot. Arrow points to the site of perforation of the infarcted area. The massive necrosis of the inferior half of the left ventricle is represented by the yellowish gray, parboiled appearance. Note that a papillary muscle is also involved.

because the major portion was clotted and adherent to the heart on all aspects. About 100 cc. of liquid blood was present.

"On the lateral aspect and at the junction of the middle and lower thirds of the left ventricle an irregular defect or tear was seen on the epicardial surface of the heart. Section through this area revealed an irregular, necrotic and hemorrhagic tear through the myocardium (Fig. 1). The myocardium at this

point presented a mottled, yellowish red, parboiled appearance, was flabby in consistency, and was obviously the seat of infarction. The rupture of the ventricle was of a subacute nature, since much of the adherent blood showed evidence of early organization upon microscopic examination.

"The coronary arteries were markedly sclerotic and the left circumflex branch was completely occluded by an adherent mottled, red and gray thrombus 5 mm. from its origin.

"On the endocardial aspect of the infarcted area of the left ventricle there was an adherent mural thrombus. The left auricular appendage also contained an adherent thrombus enmeshed between the pectinate muscles. Either of these thrombi could have been responsible for embolic phenomena.

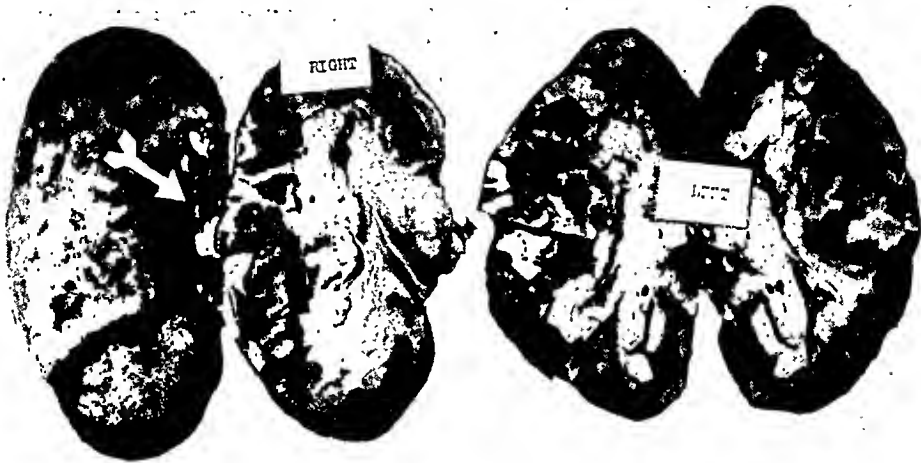


FIG. 2. Photograph of kidneys to show extent of infarction. The arrow points to the right main renal artery, which is completely occluded. The right kidney is estimated to be 80 per cent infarcted and the left 90 per cent.

"The right main renal artery (Fig. 2) was completely occluded by a tightly-wedged, red, embolic mass. The right kidney showed multiple extensive, anemic infarcts, which were pale yellowish gray in color, lying adjacent to one another and at times overlapping. Perhaps 80 per cent of this kidney was so involved. Only the lower pole was relatively free, but even this showed a hyperemic appearance, due to the terminal complete occlusion of the right main renal artery. At best, this patient had only about 20 per cent of functional right kidney before the terminal occlusion of the right main renal artery.

"At the mouth of the left main renal artery a loosely adherent embolic mass was found, which dropped out upon handling. The branches at the hilus, however, were tightly plugged by embolic masses. The left kidney was essentially similar to the right, but here the infarcts were larger and approximately 90 per cent of this kidney was the seat of ischemic necrosis.

"A small anemic infarct was found in the upper pole of the spleen.

"Starting at a distance 8 cm. above the site of femoral embolectomy, the left iliac, femoral, popliteal, anterior and posterior tibial arteries were completely

occluded. It was impossible to state whether this occlusion was due in part to emboli or postoperative thrombosis. No distinct embolic masses could be found in these situations or in the dorsalis pedis artery. The line of demarcation or of ischemic necrosis in the amputated leg was not great and consisted of a violaceous area of discoloration about the great toe. The balance of the leg showed only pallor and mottling.

"Bilateral, terminal bronchopneumonia was present."

Anatomic Diagnoses

(1) Coronary arteriosclerosis, with recent thrombosis of left circumflex artery; (2) recent massive myocardial infarction of lateral wall of left ventricle, with subacute perforation of wall; (3) organizing hemopericardium (100 cc. plus); (4) organizing mural thrombi left ventricle and left auricle; (5) embolic occlusion of both main renal arteries with massive multiple infarctions of both kidneys; (6) infarction of spleen; (7) bronchopneumonia, bilateral; (8) recent embolectomy of left femoral and popliteal arteries; (9) recent mid thigh amputation, left leg; (10) thrombosis (postoperative) left iliac, femoral and popliteal arteries; (11) cardiac hypertrophy.

Dr. Simon. "This case, therefore, from an anatomic point of view, is a straightforward case of coronary thrombosis with myocardial infarction. In the area of infarction a mural thrombus formed, as is so frequently the case, and from such a thrombus emboli resulted.

"This patient's clinical course was marked by embolic phenomena to kidneys, left lower extremity and spleen. In the kidneys the ischemic necrosis was so extensive as to lead to oliguria and uremia.

"Additional features of anatomic and clinical interest in this case are the facts that the area of myocardial infarction proceeded to subacute perforation, with a resulting organizing hemopericardium. Also interesting is the fact that a thrombus was found in the left auricular appendage, which could also have been a source for embolic phenomena.

"It is of importance to remember that massive infarction of kidneys can occur without the presence of red blood cells in the urine."

Dr. Kaufmann. "Unfortunately, this patient did not have a blood non-protein nitrogen determination upon admission. I had originally planned to amputate the leg on the day before he died, but when I discovered that the blood nonprotein nitrogen was 223 mg. per 100 ml., I felt that the case warranted further clarification. Frankly, I must say that I have never seen gangrene of the extent that this patient had, or for that matter, even massive gangrene of an extremity associated with renal impairment of this degree."

Dr. A. Stillman. Five months ago, when I performed a prostatectomy upon this man, his blood nonprotein nitrogen was normal."

Dr. Segall. "If we had taken the possibility of renal infarction more seriously, we would have reconsidered the necessity for amputation."

Dr. Simon. "I assume that the justification for amputation lay in the interpretation that the absorption of toxic products from the ischemic leg threw a

burden on the kidneys of this patient to a degree to produce uremia. Under such circumstances, one would have to invoke a pathogenesis similar to that encountered in so-called 'lower nephron nephrosis'. No lesions of the lower nephron or heme casts were seen in any of the kidney sections microscopically. I would agree with Dr. Kaufmann that I have seen much more extensive ischemic necrosis of extremities than was present in this case, without uremia being produced."

Dr. H. A. Baron. "What is the incidence of rupture of the myocardium in myocardial infarction in this hospital, and was the diagnosis made in this case?"

Dr. Simon. "I cannot give the exact figures at the moment, but I would say that it is approximately 2 per cent."

Dr. Baron. "Also, was Dicumarol indicated in the present case?"

Dr. Segall. "The use of Dicumarol in coronary thrombosis has been studied for several years, and we have not yet decided in what group of patients we should give Dicumarol routinely. Where there is good evidence of an endocardial lesion, it is perhaps wise to give Dicumarol, because on top of such a lesion thrombi may form and emboli may be thrown off.

"In regard to the recognition of hemopericardium clinically, it may be that if this man had been more fully conscious during the last twenty-four hours of his life he might have had extreme pain to draw our attention to it."

EDITORIAL

THE PAPANICOLAOU METHOD

The pathologist is a born skeptic. It seems natural for him to accept nothing until it is thoroughly established and so he has adopted this attitude toward the method of cancer diagnosis advocated by Papanicolaou.

In September, about seventy of us who were interested in Dr. Papanicolaou's method met for two weeks with him in his laboratory at Cornell Medical School. He made available for us a large amount of material which had been checked carefully. He had several outside speakers including representatives from the Cornell group, from Memorial Hospital and from some hospitals outside the city. They gave their experiences and, without exception, they felt that the technic is a definite diagnostic aid in the early diagnosis of cancer. In cancer of the stomach and cancer of the lungs, roentgenography usually reveals the tumor after it has developed too far to obtain good surgical results. Although cancer of the uterine cervix may be visible, the uterine fundus is invisible and frequently cancer can develop there without early detection. Dr. Papanicolaou, Dr. J. E. Ayre of Montreal, Dr. Joe Meigs, Dr. Shields Warren and Dr. Olive Gates of Boston, and others have found this a valuable aid in early diagnosis of cancer.

Dr. Papanicolaou will be the first to admit that the technic is really not new but he has contributed renewed study and has shown that cytology is effective even before histology in many carcinomas. Tissue sections will always be needed as a final check but Dr. Papanicolaou's work seems to indicate that often the earliest changes in carcinoma are reflected in the nuclear structure in the very superficial or exfoliated cells. He has tried many stains and the one he now uses shows good nuclear detail in the exfoliated cells. The stain must be light enough so that the cytoplasm of the cell remains transparent and the nuclear detail becomes visible. Those who have had wide experience with this technic have detected many tumors by this method before they were demonstrated by other studies. During the course with Dr. Papanicolaou, he acted as coach in the hope that he could pass the ball to others in this game of diagnosis. Dr. Papanicolaou is distinctly modest in his claims for the technic. It must be admitted that the method is time-consuming, requires considerable experience and skill and is attended by possible errors in diagnosis. Despite these limitations the method possesses too much value to be summarily dismissed.

Youngstown Hospital Association
Youngstown 1, Ohio

HORACE K. GIFFEN, M.D.

SELECTED ABSTRACTS

osteoid Osteoma. Review of the Literature and Report of Thirty Cases. MARY S. SHERMAN, M. D. J. Bone and Joint Surg., 29: 918-929, October, 1947.

The author reviewed 158 cases of osteoid osteoma, 128 from the literature and 30 previously unreported cases observed at the University of Chicago.

The clinical features of the lesion are as follows. Osteoid osteoma is more common in males by about 2 to 1. Only one case has been reported in a Negro. The lesion has a predilection for adolescents and young adults. Involvement of all bones except the scapula, clavicle and cranium have been reported, and in any given bone the lesion may be entirely in cancellous bone, beneath or in the cortex, or even located subperiosteally. In the author's series, 25 per cent occurred in the spine. All patients complained of localized pain and the chief physical finding was that of tenderness. There may be palpable thickening of the bone and soft tissue swelling. This area, however, is rarely warm and never red. There are no systemic symptoms or findings.

The radiographic picture of a mature lesion is characteristic. The active nidus is usually a small round or oval area of reduced density. About the nidus is a thick, dense shadow of sclerotic regional bone, and there may be a small dense shadow within. The circumference of the shaft may be so greatly increased and the bone so sclerotic that the nidus is difficult to demonstrate by roentgenogram.

Proper treatment is excision of the nidus, but it is not necessary to remove all of the hypertrophic bone. Incomplete excision of the nidus usually results in persistence or recurrence of symptoms. Since only 8 of 155 patients were older than 30, the author assumes that the lesion may heal spontaneously. She reports one case in which a typical lesion clinically and roentgenographically was present in the left tibia of an 18 year old boy. The patient refused surgery. Twenty-four years later pain and tenderness were absent; radiographically, there was persistent sclerosis and thickening but no evidence of the nidus.

Histologically, the lesion is characterized by loose, richly vascular fibrous tissue making up the central nidus in which there are numerous giant cells and irregular interlacing trabeculae of osteoid tissue. Within the osteoid tissue are tiny fragments of irregular new bone. At the periphery of the nidus the regional bone is hypertrophic with a moderately fibrous marrow. Beyond this is completely normal bone.

The author feels that in view of the known propensity of patients to relate all skeletal disorders to some type of injury, and in view of the small number of such histories related to osteoid osteomas, trauma may be disregarded as an etiologic factor. The evidence also points against infection as a causative factor. There are no systemic manifestations; when swelling is present the area is seldom warm and never red; the lesions are always solitary; after operation the wounds heal by first intention; the few positive cultures of the lesions that have been reported have usually been a common contaminant. After consideration of all the facts the author agrees with Jaffe that osteoid osteoma probably is best interpreted as a benign tumor.

The article is an excellent concise review of the subject, illustrated by very good photomicrographs.

Detroit

OSBORNE A. BRINES

Parasitology. Examination of bone marrow in parasitic diseases.

Sternal marrow appears to be the material of choice in searching for certain parasites. Chung (Chinese M. J., 54: 397, 1938) reported 171 cases of kala-azar diagnosed by sternal puncture, and recently Loder (Proc. New York State Assoc. Pub. Health Lab., 27: 9, 1947) reported establishing the same diagnosis in a returned soldier by using this method. Chung claims that examination of the sternal marrow gives a much higher percentage of positive results than by splenic or hepatic puncture.

Rumball and Parsons-Smith (Lancet, 2: 468, 1943) and Yu and Ying (Chinese M. J., 61: 31, 1943) find malarial parasites in sternal marrow more readily than in the blood, while Linhard (Trop. Dis. Bull., 41: 14, 1944) finds trypanosomes almost twice as often in the marrow as in blood. Iams and Kieth demonstrated histoplasma in the sternal marrow of their patient (J. Pediat., 30: 123, 1947). It would seem that one would not be justified in giving a negative report where certain parasitic diseases are suspected until the marrow had been examined.

Rochester, New York.

W. S. THOMAS

The Lymphoid Tissue and Antibody Formation. J. B. MURPHY AND E. STURN. Proc. Soc. Exper. Biol. and Med., 66: 303-307, 1947.

The relationship of lymphoid tissue to antibody formation has received considerable attention in the last few years. The above authors, with Dougherty and White, are among the foremost investigators in demonstrating that antibodies are formed in lymphoid tissue and released from lymphocytes by shedding of the cytoplasm. Depletion of lymphoid tissue by x-ray prevents the development of antibodies. Injection of adrenal cortical extract increases antibody content of the blood by the destructive action of the hormone on lymphocytes. Following the removal of the adrenal glands, the lymphoid tissue of an animal undergoes extreme hypertrophy and this results in an increased number of lymphocytes in the circulating blood. Such animals show higher antibody formation than do control non-adrenalectomized subjects. Adrenalectomized animals treated with adrenal cortical hormone and then immunized to horse serum showed no significant difference in antibody response when compared to controls.

Brooklyn

LEO M. MEYER

In Vitro Study of Bone Marrow. I and II. CLAUS MUNK PLUM. Blood, Special Issue, 1: 33-63, 1947.

In the first paper, the author describes the principles and apparatus utilized for the study of human and animal bone marrow *in vitro*. The method is based to a large extent on the one presented by Osgood in 1936. Data on erythropoiesis are presented in the second article. The effect of Ringer's solution, liver extract and plasma on the development of red blood cells were studied in rabbit, cat, dog and human bone marrow. Liver extract and plasma enhance the production of erythrocytes, with greater production in the anemic subjects. The most interesting feature in the second paper, however, is the observation that erythropoiesis apparently takes place in two ways, *viz.*, (1) mitosis of nucleated red cells up to the normoblastic stage and (2) budding or "gemmation" of cytoplasm on the normoblast with the production of about one hundred non-nucleated red cells, after which the parent cell dies. The addition of colchicine to bone marrow cultures, which inhibits mitosis of nucleated cells, did not prevent the production of erythrocytes in the usual numbers.

LEO M. MEYER

Carcinogenic Properties of Radioactive Fission Products and of Plutonium. H. LISCO, M. P. FINKEL AND A. M. BRUES. Radiology, 49: 361-1947.

This report briefly summarizes the results of work carried out under the "Manhattan Project" and is concerned with the carcinogenic properties of radioactive elements produced in the course of fission of uranium. The late effects of internal (intravenous, subcutaneous, oral) administration of plutonium and of the radioactive isotopes of strontium, yttrium and cerium were followed in mice, rats and rabbits.

Single or repeated administrations of these fission products led to development of a variety of malignant tumor, the latent period of which increased with decreasing dosage. Radiostrontium, radio-yttrium and radiocerium, injected parenterally, produced osteogenic sarcomas localized predominantly in long bones. Plutonium likewise induced bone tumors but these were mainly found in the spine. Furthermore, subcutaneous injection of

plutonium or of radio-yttrium often elicited local fibrosarcomas. Radio-yttrium fed to rats resulted in development of adenocarcinomas of the colon. No true liver tumors were observed in the treated animals, in spite of high isotope concentrations in this organ. Liver damage frequently occurred, however, and regenerative processes produced small adenomas.

The great importance of this work need hardly be pointed out: Carcinogenic properties are demonstrated to be among the health hazards of atomic fission products and thus the challenge for prevention of a new category of occupational cancer is presented. On the other hand, radio-isotopes may well furnish a valuable tool for the study of carcinogenic mechanisms and related problems.

Chicago

KURT STERN

Lysis in vitro of Sensitized Leucocytes by Ragweed Antigen. T. L. SQUIER AND H. J. LEE. *J. Allergy*, 18: 156-163, 1947.

Patients clinically sensitive to ragweed pollen and with positive skin tests to the pollen antigen were used in this study. Polymorphonuclear leukocytes were added to the heparinized blood of these persons, counts of cells being made prior to the incubation and at intervals thereafter. *In vitro* lysis resulted in a loss of approximately 43 per cent of the leukocytes which had been in contact with ragweed antigen in the test tube. Inactivation of the blood by heating to 56 C. inhibited lysis probably by inactivating the antibodies in the plasma. The blood of patients adequately treated by injections of ragweed pollen extract did not show any loss of leukocytes after incubation with the antigen.

These experiments raise the question of the relation of such lysis to the leukopenia seen in some infections. If the reaction of antigen with antibody in blood produces lysis *in vitro*, the leukocyte count in some infections may reflect the amount of destruction of cells due, not to toxins altogether, but to the allergic reaction itself.

Dallas, Texas

J. H. BLACK

The Weltmann Reaction as a Diagnostic Aid. L. O. DUTTON. *Ann. Allergy*, 5: 245-246, 1947.

The author states that this test, in his work, "has become the most dependable single indicator of an inflammatory disease which we have at our disposal". It is simple, requires only the equipment found in any small laboratory and the technic is uncomplicated. He recommends it enthusiastically.

J. H. BLACK

Differences During Dicumarol Therapy in the Quick and Russell Viper Venom Methods for Prothrombin Determination. SLOAN J. WILSON. *Proc. Soc. Exper. Biol. and Med.*, 66: 126-128, 1947.

The quantitative values of prothrombin as determined by the method of Quick, using rabbit brain thromboplastin, can be correlated with dicumarol therapy and clinical hemorrhagic tendencies. The levels of prothrombin as determined by the Russell viper venom modification of Quick's method cannot at all times be correlated with the clinical state of the patient and the dicumarol therapy.

A case is cited of a patient with thrombophlebitis and pulmonary emboli who died in a state of hemorrhagic diathesis during dicoumarol therapy. An average daily dose of 240 mg. of dicumarol administered for fourteen days had failed to decrease the prothrombin to the desired therapeutic level as measured by the Russell method (viper venom as a thromboplastin). The results obtained by both methods are compared in seven patients receiving dicumarol therapy.

Chicago

BEN FISHER

Staining of Embryonic and Small Mammalian Skeletal Systems. ROBERT M. TRUE. *Stain Technology*, 22, 107-108, 1947.

A method is described for staining small vertebrate skeletons, especially developed to bring out the presence of developing bone. A clear muscle tissue is produced so that the

skeletal elements are sharply outlined. Dilute alizarin red S in 2 per cent aqueous potassium hydroxide is used, and the specimen is cleared in graded glycerins. The soft tissues are transparent and unstained, and the osseous elements are red.

BEN FISHER

Components of the Prothrombin Complex. A. J. QUICK. *Am. J. Physiol.*, **151**: 63-69, 1947.

Normal human plasma contains a fixed amount of Quick's "component B", the conventional prothrombin of the classical theory of blood coagulation; its concentration chiefly determines the prothrombin time (by the one-stage technic). In addition there is an air-labile factor (formerly Quick's "component A") and a new "component A" which, when deficient, produces a hypoprothrombinemia and bleeding diathesis that is clinically indistinguishable from any other type of hypoprothrombinemia.

The prothrombin time by the one-stage technic normally measures component B, but a prolongation may be due to a deficiency of either component B or A. In dicumarol therapy component B is decreased; it may be that in vitamin K deficiency component A is diminished.

A study is presented showing a congenital defect of a fixed low level of component B in one family and of component A in another family.

BEN FISHER

Fatal Poisoning with Aminothiazole. ANNOTATIONS. *Lancet*, **2**: 841, 1947.

Aminothiazole is one of the antithyroid substances introduced by Astwood (1943), who also sponsored thiouracil. It produced a reduction in granular cells in 5 of 13 patients (Hims-Worth and Morgan, 1946), and was considered more liable to produce toxic reactions than thiouracil.

The first fatality is reported in a nonthyrotoxic woman (Schwob, Derobert and Malzevin, 1947) who took 50 tablets (0.1 Gm. each) to produce abortion. Laboratory findings of uremia and hypocalcemia were present during coma. Death came in a few days; hepatitis and nephritis were seen at necropsy, as were localized areas of thyroid necrosis; there was no agranulocytosis. The lethal dose, ignoring elimination by vomiting, was calculated at a little less than 0.1 Gm. per Kg. of body weight. In a nonfatal case (Gaultier, 1947), 3.6 Gm. of the drug was taken in thirteen hours, also to induce abortion. There was long-continued vomiting, but no other clinical or common laboratory abnormality. Recovery took place within forty-eight hours.

S. M. RABSON

Microscopic Examination of Teeth as Means of Identification in Forensic Medicine. G. GUSTAFSON. *J. A. D. A.*, **35**: 720-724, 1947.

Dental evidence is often utilized in the identification of bodies. Macroscopic evidence, such as decay, fillings, parodontosis, tartar, attrition and smoke coating, is useful, but unsatisfactory. The use of the striae of Retzius (produced by disturbances in enamel development) also has drawbacks. Gustafson, therefore, took advantage of Ebner's lines in the dentin, using the comparison microscope and polarized light. All the teeth of the same person will not show similar lines because of different years of development. This difficulty is overcome by comparing several teeth. An incisor and a bicuspid have corresponding lines, and the latter, in turn, has lines which correspond with a molar. In this way incisor and molar may be proved to be from the same person, although they have no directly corresponding lines. A sample batch of ten teeth, submitted by another dentist, was correctly evaluated. The criteria of parodontosis, secondary dentin and cement coating on the root, incidentally, were used to estimate the age of the individual, but these estimates were only approximate.

S. M. RABSON

BOOK REVIEWS

A Handbook for the Diagnosis of Cancer of the Uterus by the Use of Vaginal Smears. By OLIVE GATES, M. D., Pathologist, Massachusetts State Tumor Diagnosis Service; Assistant Pathologist, Pondville Hospital (Massachusetts Department of Health), and SHIELDS WARREN, M. D., Assistant Professor of Pathology, Harvard Medical School; Pathologist, New England Deaconess Hospital and New England Baptist Hospital; Reserve Consultant in Pathology to the Bureau of Medicine and Surgery, United States Navy, Captain (M.C.) USNR, 182 pp., 50 plates. Cambridge: Harvard University Press, 1947.

This monograph is a working aid for those who are interested in cytology in relation to smears from the female genital tract. The outstanding feature of this monograph is the splendid photomicrographs which illustrate the variations in the normal and the pathologic changes seen in cancer. The preface calls attention to the fact that there is no sharp dividing line between benign and malignant growths. Pathologists have relied on the patterns of growth in tissue rather than on the appearance of exfoliated cells. The authors credit Dr. J. V. Meigs with overcoming their reluctance as pathologists to base a diagnosis of malignancy on exfoliated cells. With his help they have gathered a large amount of material and analyzed it. The authors call attention to the apparent simplicity and accuracy of the method, but show that this is not an easy, sure means of diagnosis by itself. It is rather "a rigorous discipline, costly in time and labor, but a method which may bear much fruit if properly used".

Chapter I enters into a general discussion of the vaginal smear method. In this the authors review briefly the results obtained by different workers and conclude in their summary that "there is no doubt that the malignant characteristics are sometimes more conspicuous in isolated cells than in sections of tissue". Whether the practicability of the vaginal smear method as a screening test can be established depends on numerous factors. There are difficulties with the method in any laboratory, it is time-consuming, and the full evaluation of its ability to discover early cancer is undetermined as yet. Three tables present the results obtained by the vaginal smear method by various workers who have reported in the literature. The third table summarizes the findings in the vaginal smear as an aid for demonstrating early and obscure cases of carcinoma. In the hands of all workers fairly large numbers of cases of cancer which were questionable or obscure clinically, were demonstrated primarily by this method.

The second chapter deals with the technic itself in making and staining the smears and discusses the various stains used for this study. Chapters III and IV deal with the normal morphology and histology of the vaginal smear cells and vaginal tissues. In these chapters are illustrated the various types of cells found normally. Here are discussed the functional and cyclic changes as well as the variations incident to age of the patients. Chapter V is devoted to the study of the types and variations in cancer of the uterus. Table IV is a classification of cancer of the uterus, while Table V shows incidence of carcinoma found in the Massachusetts State Tumor Service between 1930 and 1942 for each of the age periods. Chapter VI deals with the specific characteristics of malignancy as seen in the cells of the smears. This chapter begins with the statement, "Differences between benign and malignant cells are mainly matters of degree rather than of kind". Then the authors discuss the criteria of malignancy as seen in the exfoliated cells. They summarize the main characteristics of malignant cells as showing a macronucleolus, hyperchromatism and polymorphism. Chapter VII discusses the effects of radiation on cells found in the vaginal smears. The normal cells after irradiation may appear so atypical as to be mistaken for malignant cells. Other sources of error are discussed in Chapter VIII. In Chapter IX the authors give various aids in the study of the slides to avoid errors. The last chapter is devoted to an appraisal of the method by the authors with abundant references.

Following the text, there are splendid plates showing the variations seen in the cells exfoliated and the tissue from which they originate. The illustrations are entirely in black

and white but are technically excellent. The only adverse comment on these photomicrographs is that they would be even more valuable in color. To a pathologist the photomicrographs are far superior to drawings for conveying an accurate impression of the tissues and cells. Another very good aspect of the photographs is the uniformity of the magnification. Most of them are reproduced at 500 magnification, while a few are shown at 1000 or 2000 magnification for greater detail.

The handbook is a very valuable aid for those who are using the cytologic method for detecting cancer, and also for those who are interested in this new emphasis in medical diagnosis.

Youngstown, Ohio

HORACE K. GIFFEN

Calcific Disease of the Aortic Valve. A Comprehensive, Analytic Survey of Calcific Sclerosis.

By HOWARD T. KARSNER, M.D., AND SIMON KOLETSKY, M.D., Institute of Pathology, Western Reserve University and the University Hospital of Cleveland. 111 pp., 23 figs., 34 tables. \$5.00. Philadelphia: J. B. Lippincott Company, 1947.

This monograph which brings up to date our knowledge of calcific disease of the aortic valve, will undoubtedly remain the authoritative book on the subject for a long while. After a careful study of the writings of other students of the disease, and a masterly analysis of their own unique material, the authors conclude that "with only rare exceptions, calcific disease of the aortic valve is the result of rheumatic cardiac disease," and that the exceptions do not invalidate this conclusion.

In addition to the detailed study of calcific disease of the aortic valve, there is a valuable description of chronic nondeforming valvulitis with an admirable presentation of the evidence for attributing the cause of this lesion to rheumatic fever, in Chapter 5. Increased recognition of these lesions will doubtless lead to revision of our present concept of the incidence and nature of rheumatic heart disease. We are greatly in the debt of the authors for giving us the best account of chronic nondeforming valvulitis which has yet been published.

Finally, this reviewer wishes to mention very briefly a philosophic point, namely, the value of morbid anatomy in present day medical investigation. The book which is the subject of this review deals with a purely morphologic problem and uses the commonly accepted methods of gross and microscopic morbid anatomy in order to solve it. There is nothing new about the way in which the material has been collected and scrutinized but Dr. Karsner and Dr. Koletsky have gone about their task with a critical mind and, using well established methods, have given such an orderly, precise and clear presentation of their subject that their conclusions are inescapable. The whole work shows the hand of the master. In the presence of such a piece of work, who will be the first to say that gross morbid anatomy has no further part to play in the advancement of medicine?

Chicago

WILLIAM B. WARTMAN

An Introduction to Biochemistry. Ed. 3. By WILLIAM ROBERT FEARON, M.A., Sc.D., M.B., Fellow of Trinity College and Professor of Biochemistry, University of Dublin. 569 pp. \$6.00. New York: Grune and Stratton, 1947.

The third edition of this well known text is now available under the imprint of an American publisher. It embodies most of the features of earlier editions. It is evident that an effort has been made to make it of greater value for students of medicine, since, in the present edition, "more emphasis has been placed on certain subjects of special interest in clinical medicine". This is shown by increased discussion of such material as acid-base balance, energy problems and colorimetry, blood chemistry and related topics.

Much of the discussion includes material which is not commonly presented in American texts for medical students. The treatment of the chemistry of the carbohydrates, for example, is much more extensive. The chapter on "biological elements", unique in the experience of this reviewer, contains much that is of considerable interest to the student

of general biochemistry, but its values for the student of medicine may be questioned. The chapter on tissue respiration (Chapter 19), a subject not easy for the elementary student, is well written. In general, the presentations are clear and to the point.

Opinions as to the value of the presentation of the historical background of important problems to elementary students are divided. There are those who maintain that the factual material of modern biochemistry is so extensive that the student's difficulties are only increased by the historic approach. With this point of view, the reviewer cannot concur. It is, therefore, gratifying to find well-chosen references to classic discoveries in biochemistry cited together with the name of important investigators. An excellent example of this historic approach is the "summary of the history of muscular biochemistry", to which two pages are devoted, in which progress from Buchner's discovery of cell-free fermentation and zymase of yeast (1903) to Szent-Gyorgyi's isolation of crystalline actomyosin (1945) is detailed.

Differences in the nomenclature of Great Britain and of the United States are confusing. Such terms as hormoprotein, zymoprotein and autogenic protein are not customarily in use in the United States. The distinction between the commonly used British caseinogen and casein in contrast to the American casein and paracasein is explained, but the situation is hardly remedied by the suggestion of the use of the terms caseinogen and paracasein, an attempt to combine and reconcile the two systems of nomenclature. The book contains much that should be of value to the advanced student, but it is not believed that it will be widely used in this country for the elementary course in biochemistry as given to first year students in medicine.

Finally, it should be pointed out that in contrast to the texts most commonly used here, the book of Professor Fearon includes directions for many laboratory tests of importance. These are not listed in a separate section, but are presented along with the theoretic discussion of the various compounds whose detection is given. One regrets, in such presentations, the absence of any discussion of quantitative methods. In the reviewer's experience, the tendency in America is away from the qualitative procedures and toward the more satisfying (to both student and instructor) quantitative determinations.

Ann Arbor

HOWARD B. LEWIS

Histopathologic Technic. By R. D. LILLIE, A.B., M.D., Medical Director, United States Public Health Service; Chief, Pathology Laboratory, National Institute of Health. 300 pp., 14 tables. \$4.75. Philadelphia: The Blakiston Company, 1948.

This is the most up-to-date and complete text on histopathologic technic available. It contains not only practically all the useful methods published in the last few years, but also excellent surveys on the chemistry of certain tissue constituents such as the pigments and lipid substances. There is a very useful table of buffers at the end of the book. Dyes are specified by their Color Index Number to avoid confusion. The only technic enjoying considerable popularity and yet not included in the book is Papanicolaou's cytologic stain for smears. The author's practical hints and modifications should prove very valuable for those less versed in the more complicated methods.

This book is highly recommended, especially to those histologists and pathologists who are interested in microscopic technic itself, are fascinated by its whys and hows and possibilities, and do not consider it only as a means to an end. To the tissue technician who does not have the scientific background and who only wishes to find a suitable method for the staining of a histologic structure (*e.g.*, reticulum fibers), the long array of variants quoted may prove bewildering rather than helpful.

Chicago

GEORGE GOMORI

Textbook of Bacteriology. Ed. 4. By THURMAN B. RICE, M.D., Professor of Bacteriology and Public Health, The Indiana University School of Medicine. 603 pp., 127 figs. \$6.50. Philadelphia: W. B. Saunders Company, 1947.

It is the opinion of the reviewer that Rice's *Textbook of Bacteriology*, Fourth Edition, is not, in a strict sense, a textbook of bacteriology, but a series of preparatory lectures intended as an introduction to medical bacteriology for medical students. This is indicated in the author's preface where, it is stated, the book is "an introduction to an important and difficult subject which has sometimes tended to bog down from the sheer weight of its exacting technic and detail". The author throughout stresses specific diseases and their causative agents, treatment and control of infectious diseases, rather than pathogenic bacteria viewed in a botanical sense. The title of this book does not agree with its contents and the book could well be called "An Introduction to Medical Bacteriology".

If read with the author's intent in mind, it is evident Rice has succeeded in indicating salient points of medical bacteriology from which the beginner may advance to detailed texts of bacteriology in which theory, techniques, controversial issues and history are given. The book should prove a valuable adjunct in medical schools to texts such as *Topley and Wilson* and *Jordan and Burrows*. Whether it will be, as the author suggests, instrumental in improving the practical service which physicians, dentists and pharmacists will be able to give their patients seems doubtful.

Some errors are noted which perhaps are deliberate on the part of the author in his effort to give a clear picture of a confusing subject. In particular, it is implied that the causative agent of scarlet fever is a single entity to be grouped under *Streptococcus pyogenes* rather than a series of antigenic types of *Str. pyogenes*. It is stated, also, that *Proteus morganii* is frequently responsible for summer diarrheal diseases of children, without mention that the role of this organism in diarrhea is questionable.

The text includes chapters on Rickettsial diseases, diseases of virus etiology, mycology and parasitology. The inclusion in the appendices of information relative to methods for collection of specimens, regulations governing transportation of specimens through the mail, and the brief description of the regulations governing production of biologic products should be of particular interest and value.

Lansing, Michigan

H. E. COPE

Beiträge zur Kenntnis der Blutgerinnung. By W. K. RIEBEN, Professor für experimentelle Medizin an der Universität Oregon Ehem. wissenschaftlicher Mitarbeiter am Chemischen Institut der Universität Zürich. 96 pp., 26 figs., 13 tables. 9 gebunden francs. Basel, Switzerland: Benno Schwabe & Co., 1947.

In the first part of this monograph a quantitative determination of prothrombin in two phases is described and a greater accuracy is claimed for it, as against Quick's monophasic method. Oxalated blood plasma is diluted fifty times with salt solution to exclude the effect of antithrombotic substances as well as of fibrinogen. In the first phase of the author's procedure, the prothrombin is activated to thrombin by the addition of thromboplastin and calcium. When the appearance of a fine fibrin web indicates the maximum of thrombin activity, 0.2 cc. of the mixture is pipetted off, heated to 37 C. and 0.1 cc. of fibrinogen solution is added. The time between addition of fibrinogen and the appearance of a definite clot is determined and compared with that of normal plasma (90 mg. of pooled dry plasma dissolved in 1 cc. of distilled water). The prothrombin activity is reported in percentage by dividing the coagulation time of the unknown by that of the normal and multiplying by 100.

In the second part, the author justifies his modification of Quick's method by theoretical considerations and experimental data. The third part of the monograph deals with the effect of amino acids and related substances on the different phases of the clotting mechanism.

This monograph is based on careful experimental and clinical observations and is an interesting contribution to the problems of coagulation.

Wichita, Kansas

C. A. HELLWIG

Tuberkulöse Reinfektion beim Rinde und ihr Einfluss auf die Resistenz. By E. GRÄUB P.D. with W. ZSCHOKKE, P.D., E. SAXER, P.D., and VEB. H. VONARBURG. 93 pp., 18 figs., 12 tables. 12 Swiss francs. Basel, Switzerland: S. Karger, 1947.

Since 1932, observations on the course and outcome of primary and re- or superinfection tuberculosis have been conducted with a mildly virulent bovine strain (P strain) of tubercle bacilli. The first infections and later reinfections were found subcutaneously as new lymph nodes. Skin, subcutaneous tissues and muscle were only slightly susceptible to tuberculosis. In the vicinity of these tissues lie numerous lymph nodes, whose excision and examination made it possible to follow the reaction and course resulting from subcutaneous infection. A primary test period from 1932 to 1941 disclosed features of the pathogenicity of the P strain in first infection and reinfection of calves, steers and cows; the influence of infection on allergy and on the milk secretion of the infected animals; and the resistance to natural infection resulting from contact with open cases. During the second period, the resistance was tested by artificial infection with virulent tubercle bacilli. It was found that the P strain retained viability at least twelve weeks and that it was harmless subcutaneously even to young calves, producing a painless nodule. The local reaction on reinfection is similar to the first infection. After reinfection, neither anaphylactic nor other symptoms were noted. Cows in lactation were not noticeably affected whether injections occurred before or after delivery, and tubercle bacilli were never excreted in the milk. In no case following first infection or reinfection with the P strain did an endogenous infection occur; the bacilli spread only to the related lymph nodes, never beyond them. Cattle infected with P strain as calves and later reinfected as cows, were tuberculosis-free at slaughter after contact for three or more years with open cases of tuberculosis. In inoculation site or lymph nodes, living bacilli of P strain can be found after two to three years, thus permitting the deduction that increased resistance can last for years. In most cases, but not in all, first infection with the P strain produced allergy to tuberculin, but not consistently, by all routes tested (subcutaneous, intradermal or the eye). This allergy persists about one year. Later the reinfected animals were negative to subcutaneous tuberculin tests even though infected with virulent bacilli. Negatively changed intradermal and ophthalmic reactions after first infection can become positive either as a result of artificial or natural infection in some cases. As an indicator of increased resistance, lymph nodes containing tubercle bacilli living in symbiosis, are more reliable than allergy. Bacteriologically healed or residual nodes have the same significance for resistance. Saxer contributes briefly to the origin of tubercle bacilli in cow's milk. In guinea pigs in which clinically healed first infections existed (antiphymatol, Friedman bacilli, BCG, and bacilli of different virulences, there was only a slight increase in resistance over controls; while in those with reinfection and with evident tuberculous changes and enlargement of local glands there was an outstanding resistance present.

The authors' opinions may not all be valid, but the study is another step toward solving the intricate ramifications of tuberculosis and merits reading by those interested in tubercology and its implications.

Denver

H. J. CORPER

Mycopathologia. Vol. IV, 30, VIII, 1947, Fasc. 1. Edited by R. CIFERRI, Pavia, and P. REDAELLI, Milano. 84 pp., 4 figs., 2 tables. Subscription price for the volume, 36 Dutch francs. Amsterdam: Dr. W. Junk, 1947.

This journal, published by Dr. W. Junk, Amsterdam, and edited by the well known mycologists, R. Ciferri and P. Redaelli, has just made its reappearance in the scientific literature.

This issue consists of 84 pages and contains five original papers. The first article, written by Cesare Cavallero, University of Pavia, is on allergy and immunity in mycoses. The author reviews the literature on several diseases, such as dermatitis seborrheica, psoriasis and

eczema, some of which are not widely accepted as caused by fungi, and attempts to show allergic and immunologic phenomena in those diseases. He points out the necessity of host sensitization before the mycosis is produced, and concludes that hypersensitivity due to repeated contacts with the agent plays an important role in the production and course of mycoses. A large bibliography is appended.

The second paper is by Professors Redaelli and Ciferri and consists of a report of the activities of the Center of Human and Comparative Mycology of Pavia during the period 1938-1941. The Center of Mycology receives cultures of fungi from all over the world and classifies them and keeps a so-called Mycotheca which is available to anyone interested in the subject. There is listed the yeasts and fungi received during the period of four years. Two new species are described in detail, *Mycotorula messanensis*, isolated from a case of human dermatosis and *Glenospora viridobrunnea*, isolated from a granulomatous lesion on the dorsum and sole of the foot of a woman. The research which the authors have conducted in the past on the general Histoplasma, Perieystis and Cystidiella are presented.

The next paper is by Verona on the presence and number of Actinomyceetes in farm soils and their relationship to the different seasons. He found in Italian soils that the number of Actinomyceetes was greatest during the autumnal period.

The following paper by Ciferri and Redaelli deals with the isolation of *Mycoderma glutinis-farinulae* from ferment (yeast) used in bread making in Italy. In place of the generic name, *Mycoderma*, the authors propose "*Mycokluyveria*", which is dedicated to Professor K. J. Kluyver of Delft.

The last paper is written in German. The author Baldacci, presents a modern key for all families, subfamilies and genera of *Actinomyceetales Buchanan*. A few of the most important species are described and discussed from the historic and experimental point of view.

He divides the different species in groups of those that have never been cultured, in which, surprisingly, he includes *Streptothrix Foersteri*, *Actinomyces bovis* and *Actinomyces hominis*, and those which he considers valid species, such as *Actinomyces Bostroemi*, *A. albus*, *A. sulphureus* and *A. madurae*.

Madison, Wisconsin

PAULO DACORSO

The Pathology of Traumatic Injury. A General Review. By JAMES V. WILSON, M.D., Pathologist to Harrogate and District General Hospital, and the Royal Bath Hospital, Harrogate. Sometime Associate Professor of Pathology Farouk I University, Alexandria. D. A. D. P. Malta Command, 1940-1943. 192 pp., 61 figs., 10 in color. \$6.00. Baltimore: The Williams & Wilkins Company, 1946.

Wilson employs the word "pathology" in its broadest sense, including physiology as well as anatomy. The subtitle "A General Review" is the clue to the volume. Traumatic shock, burns, crush injury, fat embolism, blast injury and wounds and infection form the subject matter of half the small book, and the remainder is devoted to injuries to chest, blood vessels, abdomen, nervous system and bones and joints. The bibliography is extensive and includes items published in 1945. Strangely enough, there is no mention of Moritz's American volume on the same subject.

The atomic bomb casualties are not discussed because of the date of the book's production. "Blast injury" will have to be amplified and another chapter devoted to irradiation injury. Despite these understandable drawbacks, *The Pathology of Traumatic Injury* well deserves a place in the library of both pathologist and surgeon. It is recent and well written, although necessarily truncated.

Fort Wayne, Indiana

S. M. RABSON

Neuropathology, Its Clinicopathologic Aspects. By I. MARK SCHEINKER, M.D., Professor of Medicine (Neurology) and Instructor in Neuropathology, University of Cincinnati, College of Medicine, Neuropathologist and Attending Neurologist, Cincinnati General Hospital. 306 pp., 208 figs. \$6.75. Springfield, Illinois: Charles C Thomas, 1947.

This book on neuropathology deals with the more fundamental and frequently observed diseases of the central nervous system. It is written more for the clinician and student of neurology than for the pathologist, yet there is much that is of interest to general pathologists. Most subjects are adequately treated, but at times the author emphasizes his own views in place of well-established observations made by previous workers. Some chapters will require revision in a subsequent edition. One of the weaknesses of the book is the small size of the illustrations of gross specimens; furthermore, in many of the photomicrographs higher magnifications would be an asset.

This is the first volume of a series of three planned by the author. The second is to be devoted to neurosurgical neuropathology, and the third volume is to be on medical neuropathology.

Rochester, Minnesota

JAMES W. KERNOHAN

Brain and Body Weight in Man: Their Antecedents in Growth and Evolution. A Study in Dynamic Somatometry. By EARL W. COUNT. 129 pp., 24 figs., 31 tables. \$2.00. New York: The New York Academy of Sciences, 1947.

On the basis of ontogenetic and phylogenetic data, the following conclusions are made. The growth of brain weight with respect to body weight has three periods: straight-lined periods in fetal and in life after infancy, and a transitional period through infancy marked by a curved line. During the time of curvilinear growth, mitoses of brain cells diminish to none. In postinfantile life, the rise of brain weight, relative to body weight is less in man than in the monkey. The human brain, during the measurable fetal period, does not grow much more rapidly than the monkey's. Phyletic increase in complexity of cerebral organization requires phyletic increase in absolute brain size, contingent upon phyletic increase in total body size. Contrary to Dubois' system, the logarithm of brain weight traces against the logarithm of body weight an ascending curve, concave on the lower side, and expressible as a second-degree parabola, i.e., the "exponent of cephalization". The reptilian cephalization exponent is an ascending curve, concave on its upper side. The mammalian step-up in the proportion of body weight devoted to brain is largely confined to those portions of brain practically unrepresented in reptiles. From cercopithecoid to homo is less of a stadium than from hapalid to cebid or from insectivore to hapalid. The primate has put more material into the weight of brain than has any other mammal. As to the parallelism between fetus and comparative anatomy, a fetus of a given body size always has a heavier brain than some extant adult relative of equal body size, who is less evolved. A principle underlying all mammalian cephalization is that increase in absolute body size is a requisite for higher cephalization.

Detroit

GABRIEL STEINER

Physical Fitness Appraisal and Guidance. By THOMAS KIRK CURETON, JR., M.A., M.P.E., Ph.D., assisted by FREDERICK W. KASCH, B.S., M.S., JOHN BROWN, M.S., and W. G. MOSS, M.S., Ph.D. 558 pp., 66 figs., 100 tables. St. Louis: C. V. Mosby Company, 1947.

This very interesting book relates the experiences of the physical education staff of the University of Illinois in attempting to establish methods for making physical fitness appraisals. Most of the data is based on observations made upon the entering students on both the Urbana and Chicago campuses of the University.

The writers do not define exactly what physical fitness is, but indicate that it is one phase of total fitness and is concerned with appraisals of the physique, the organic efficiency and the motor fitness of the individual. Extensive studies are reported, dealing with body type and with tabulations of measurements. Other studies deal with functional tests and responses to many kinds of effort. These tables are very comprehensive, but by the very nature of the material studied, the findings are applicable largely to young people of college age, although some studies are based on observations on 100 middle aged men.

An appraisal chart for rating physical fitness is proposed with factors to be rated under

physique, organic and motor fitness phases. A large number of tests are proposed in this scheme.

This book should be of interest to all those faced with the problems of appraising physical fitness. The complete and careful way in which the data have been compiled should help to standardize methods for such appraisals.

Detroit

BRUCE H. DOUGLAS

A Textbook of Medicine. Ed. 7. Edited by RUSSELL L. CECIL, M.D., Sc.D., Professor of Clinical Medicine, Cornell University Medical College, with the assistance of WALSH McDERMOTT, M.D., Associate Professor of Medicine, Cornell University Medical College, Associate Editor for Diseases of the Nervous System, HAROLD G. WOLFF, M.D., Associate Professor of Neurology, Cornell University Medical College. 1730 pp., 242 figs. \$10.00. Philadelphia: W. B. Saunders Company, 1947.

With the seventh edition of his textbook of medicine, Cecil presents a revised and most complete medical reference text of inestimable value to the student and practitioner of medicine. The 162 contributors to this book are authorities in their individual fields and present their subjects in a refreshingly concise style. The name of the contributor is added at the end of each article. It is understood that with a text of such scope detail must be sacrificed in many instances for the broad picture of the disease entity. Included among the subdivisions of each disease are paragraphs on etiology and morbid anatomy, and in some instances, on bacteriology, clinical pathology, pathologic physiology and chemistry. A list of eight or ten references to the pertinent literature is appended.

This edition contains a number of new discussions on subjects not previously presented, including blackwater fever, drug allergy, marijuana intoxication, vitamin deficiencies (A, E, K), hypervitaminosis, acrodynia, porphyria, hemoglobinurias, diphtheritic polyneuritis, psychosomatic medicine, hemifacial spasm and narcolepsy.

Sections which should be of particular interest to readers of the Journal include the following: diseases of the spleen and diseases of the reticulo-endothelial system by E. B. Krumphaar; radium poisoning by H. S. Martland; parasitic infections by E. C. Faust; trench fever and Carrión's disease by H. Pinkerton; and rickettsial diseases, typhus fever and Rocky Mountain spotted fever by S. B. Wolbach. There is also an appendix on "normal values for clinical examination" by Ralph G. Stillman.

Many of the black and white prints have lost some detail in reproduction, but the illustrations in color are of good quality. As each new edition of this book appears, it becomes more of a medical classic and more necessary to the physician's medical library.

Detroit

MARK DALE

History of Medicine. By CECILIA C. METTLER, A. B., Ed.B., A.M., Ph.D., Late Assistant Professor of Medical History, University of Georgia School of Medicine and Late Associate in Neurology, College of Physicians and Surgeons, Columbia University. Edited by Fred A. Mettler, A.M., M.D., Ph.D., Associate Professor of Anatomy, College of Physicians and Surgeons, Columbia University. 1213 pp., 16 illus. \$8.50. Philadelphia: The Blakiston Company, 1947.

This book cannot be better reviewed than in the statements that follow, written by a nationally-known scientist, Dr. Olaf Larsell, himself a serious student of medical history:

"This book has been written with the purpose of providing a text for the medical student and the teacher of medical history who is not a professional historian, but who has a serious interest in the history of medicine. The material is organized by subjects and is arranged so that the various subjects appear in approximately the order in which they are presented in the medical curriculum of today, the older subjects being carried to about the middle of the nineteenth century.

"Sufficient biographical detail is included regarding the chief contributors to the growth of the various fields to give some notion of their personalities as well as their specific contributions to knowledge or teaching.

"There is an excellent index and the subject matter is documented with numerous references to original sources, which the author has consulted in the original.

"Editing of the book has been well done and the publishers have produced an attractive and substantial volume which will be a valuable addition to the library of every student of medical history."

The fifteen subjects arranged by specialties in medicine from "Anatomy" through "Otolaryngology" will be particularly appreciated by pathologists. Illustrations are few but are unusually well chosen and consist of excellent plates at the beginning of each chapter. Names are in bold type and biographic dates follow each name. Source references are cited on the same page as the text which they support, making the book much easier to use. An extensive 110-page Subject and Personal Names index is also very attractive to the reader who appreciates ready accessibility of source material.

Portland, Oregon

FRANK B. QUEEN

Diseases of Metabolism, Detailed Methods of Diagnosis and Treatment, A Text for the Practitioner. Ed. 2. By GARFIELD G. DUNCAN, M. D., Director of Medical Division Pennsylvania Hospital; Clinical Professor of Medicine, Jefferson Medical College, Philadelphia, Pennsylvania. 1045 pp., 167 figs. \$12.00. Philadelphia and London: W. B. Saunders Company, 1947.

In the second edition of this book six additional authors have been added to the list of fifteen who contributed to the first edition. Two new chapters are included, one on Disorders of the Thyroid Gland and one on Diseases of the Kidney. Both of these new chapters are well written; however, the propriety of inserting chapters on diseases of special organs in a book on metabolism may be questioned by some of our readers. Admittedly, the field of metabolism has expanded to such an extent that it is difficult to define the limits to which it might be restricted. As an example, in the excellent chapter on disorders of the blood, the author, Leandro M. Toeantins, points out that the diagnosis of blood diseases in the past has been considered primarily as a morphologic problem approached by standard hematologic methods; with increasing knowledge, however, it has become apparent that the principal causes of some of these disorders are often related to nutritional deficiencies. Therefore, the subject of blood disorders must now be considered also from the viewpoint of defects of body metabolism.

The scope of the book perhaps can be indicated best by mentioning chapter headings and the responsible authors for the various chapters: Introductory Considerations, Hyperinsulinism, Diabetes Insipidus, Diabetes Mellitus by Garfield G. Duncan; Carbohydrate Metabolism by C. N. H. Long; Protein Metabolism, Lipid Metabolism by Abraham White; Mineral Metabolism, Melituria by Abraham Cantarow; Water Balance in Health and Disease by John P. Peters; Nutritional and Metabolic Aspects of Disorders of the Blood by Leandro M. Toeantins; Vitamins and Avitaminosis by Tom D. Spies and Hugh R. Butt; Undernutrition by L. H. Newburgh; Obesity by Frank A. Evans; Xanthomatosis, Glycogen Disease, and Disturbances of Intermediary Metabolism by Edward Mason; Gout by Walter Bauer and Friedrich Klemperer; Disorders of the Thyroid Gland by Alexander W. Winkler; and Diseases of the Kidney by Max Miller and Joseph M. Hayman, Jr.

Philadelphia

F. WILLIAM SUNDERMAN

The American Hospital. By E. H. L. CORWIN, Ph.D., Executive Secretary, Committee on Public Health Relations, The New York Academy of Medicine; Honorary Charter Fellow of the American College of Hospital Administrators; former Secretary General and Honorary President International Hospital Association. 226 pp., 2 figs., 23 tables. \$1.50. New York: The Commonwealth Fund, 1946.

The American Hospital is a large title for a small book. It crowds into its 226 pages a considerable number of facts and figures which are intelligently discussed and ably analyzed.

The author, who is an authority on hospital service, recognizes the rapid economic and social changes and the fundamental advances in medical science. A few of the many important points which are made may be mentioned. General hospitals furnish 40 per cent of the total bed capacity and are used by 90 per cent of the people who go to hospitals. They create many new advances in medicine and set the pace for high standards. About 70 per cent of the indigent sick are in nongovernmental hospitals, most of them in mental hospitals. Municipal, county, state and federal governments in recent years have increasingly made use of beds in voluntary hospitals for the acute and chronic sick and for maternity service especially for wives of military personnel. The optimum size of a hospital is from 200 to 500 beds. Rising operating costs are a concern of those who cannot afford to pay for them and yet are not eligible for ward service. Hospital prepayment insurance schemes have been economically helpful to a large number of people. About 75 per cent of all sources of hospital income is derived from patients. Endowment funds of all voluntary hospitals are under 500 million dollars, and the income thereof furnishes only about 6 per cent of all hospital income, while philanthropy meets the deficit. Hospitals are unevenly distributed in several parts of the country. The postwar hospital building needs for general, tuberculous and mental patients is 2 billion dollars or 200 million dollars a year for 10 years.

Dr. Corwin has not discussed the trend and the need of making dental service a significant clinical function of the hospital and outpatient service. He might have discussed more fully the trend toward group medical practice and its effect on medical practice and hospitals; also the profound effect on the hospital of organized home medical care and of rehabilitation of patients.

The monograph is a significant contribution. It adds in a clear way to the record of hospital conditions as they exist today and the trends and forces which compel it to adjust itself to the newer conditions and ever-changing needs of the average American community.

New York

J. J. GOLUB

Practical Office Gynecology. By KARL JOHN KARNAKY, M.D., Assistant Professor of Clinical Gynecology, Baylor University College of Medicine; Gynecologist to Jefferson Davis Hospital; Director of Menstrual Disorder Clinic, Jefferson Davis Hospital, Houston, Texas. 261 pp., 113 figs. \$7.50. Springfield, Illinois: Charles C Thomas, 1947.

The author of *Practical Office Gynecology* is well-known among gynecologists as an energetic, enthusiastic worker and a voluminous writer whose investigations provide the basis for most of the procedures advocated in the text. It contains many original ideas which are either stimulating or, most certainly, provocative. The many illustrations are for the most part excellent.

While one must agree with the author that the ever-widening scope of medical gynecology justifies increasing emphasis upon non-surgical procedures in this field, many of his suggestions have not been sufficiently established to warrant acceptance without further proof. An example of this is his unbridled enthusiasm for huge doses of stilbestrol as a panacea for a wide variety of gynecologic disorders. Proof that his form of therapy is either efficacious or entirely innocuous is not convincingly demonstrated in the text. Great harm might result from wide-spread acceptance of such advice by uncritical readers. The chapter on "Protozoal, Fungal and Spirillar Infection of the Female Genital Tract", by all odds the best feature of the book, contains excellent illustrations.

It is regrettable that little can be said in praise of this work. It appears to have been hastily written and carelessly edited, as is apparent from the many typographical and grammatical errors and from its generally poor style and composition. This is all the more deplorable since no expense has been spared to provide the materials for what might have been an acceptable publication.

BOOKS RECEIVED

Books received are acknowledged in this column, and such acknowledgment must be regarded as sufficient return for the courtesy of the sender. Selections will be made for more extensive review in the interests of our readers and as space permits.

- L'Hyperinsulinie. Les États de Suractivité Fonctionnelle du Pancréas Endocrin en Médecine Expérimentale et en Clinique.* MARCEL SENDRAIL, Professeur de Pathologie Générale à l'Université de Toulouse. 256 pp., 25 figs., 2 color plates. 500 francs. Paris: Masson et Cie, 1947.
- Nouvelles Études Cliniques et Biologiques sur la Pathologie du Foie.* ÉTIENNE CHABROL, Professeur de Clinique Médicale à la Faculté de Paris, Membre de l'Académie de Médecine. 182 pp., 24 figs., 34 tables. Paris: Masson et Cie, 1946.
- Archivio di Fisiologia*, Volume 11, Number 1, January, February, 1947. 95 pp., 21 figs.
- L'Organisme en Lutte Contre Les Microbes.* ANDRÉ BOIVIN, Chef de Service à l'Institut Pasteur, Membre de l'Académie de Médecine, and Albert DeLaunay, Chef de Laboratoire à l'Institut Pasteur. 425 pp., 15 figs. Paris: Librairie Gallimard, 1947.
- Hematologica Archivio Fondato da Adolfo Ferrata*, Vol. XXIX, 1946, Fasc. I-III. 232 pp., 28 figs.
- Archivos Del Hospital Santo Tomas*, Volume 2, Number 1, January, February, March 1946. 143 pp., 52 figs.
- Clinique et Investigations.* NOEL FIESSINGER, Professeur de Clinique Médicale à l'Hôtel-Dieu, Membre de l'Académie de Médecine. 892 pp., 192 figs. Paris: Masson et Cie, 1946.
- Some Aspects of Red Cell Production and Destruction.* ERIC PONDER, WILLIAM B. CASTLE, HARRY A. CHARIPPER, WILLIAM DAMESHEK, ALBERT S. GORDON, S. GRANICK, AND F. S. ROBSCHIEIT-ROBBINS. 128 pp., 13 figs., 3 plates, 19 tables. \$2.00. New York: The New York Academy of Sciences, 1947.
- Synopsis of Obstetrics*, Ed. 3. JENNINGS C. LITZENBERG, B. Sc., M.D., F.A.C.S., Professor Emeritus of Obstetrics and Gynecology, University of Minnesota, Medical School. 416 pp., 157 illus., 5 in color. \$5.50. St. Louis: C. V. Mosby Company, 1947.
- Arteriosclerosis de Las Extremidades.* DR. F. MARTORELL, Jefe de la Sección de Cirugía Vascular del Instituto Policlínico de Barcelona. 107 pp., 10 figs. Barcelona: Colección Española de Monografías Médicas, 1947.
- Meningitis Meningococcica en la Infancia (Meningitis Cerebroespinal Epidémica.* DR. WALTER STIRNIMANN. 67 pp., 13 figs., 1 table. Barcelona: Colección Española de Monografías Médicas, 1947.
- Tumores y Seudotumores de la Mama. Estudio de Investigación Experimental su Profilaxis y Tratamiento.* DR. JACINTO MOREÑO. 142 pp., 40 figs. Buenos Aires: Lopez and Etchegoyen, S. R. L., 1946.
- Zur Chemotherapie der Sulfone Gegen Tuberkulose. Experimentelle Untersuchungen am Meeresschweinchen unter klinische Erfahrungen beim Menschen mit der 4,4'Diaminodiphenylsulfon-Glukose-Bisulfiterbindung.* O. ACKLIN, E. ROSSI AND M. SCHMID. 25 pp., 6 figs. 2 broschiert francs. Basel: Benno Schwabe and Company, 1946.
- Metallschädigung bei Osteosynthesen Experimentelle und Klinische Untersuchungen über Wesen und Bedeutung der Metallose und der Korrosion.* R. NICOLE, Sekundärarzt der Klinik. 74 pp., 42 figs., 6 tables. 8 broschiert francs. Basel: Benno Schwabe and Company, 1947.
- The Physico-Chemical Mechanism of Nerve Activity.* DAVID NACHMANSOHN, CHARLES M. BERRY, OSCAR BODANSKY, FRANK BRINK, JR., DETLEV W. BRONK, M. VERTNER BROWN, C. W. COATES, R. T. COX, J. C. ECCLES, ALFRED FESSARD, J. F. FULTON, R. W. GERARD, ALFRED GILMAN, D. E. GREEN, JOSEPH C. HINSEY, RUDOLPH HOBER, MARTIN G. LARRA-

- BEE and TRACY J. PUTNAM. 227 pp., 53 figs., 5 plates, 9 tables. New York: New York Academy of Sciences, 1946.
- Folic Acid Supplement, the Synthesis of Pteroylglutamic Acid (Liver L. Casei) Factor and Pteric Acid-Part II.* M. E. HULTQUIST *et al.* Vol. XLVIII, (Art. 5). pp. i-vi. New York: New York Academy of Sciences, 1947.
- Transactions of the New York Academy of Sciences*, January 1947, Ser. II, Vol. 9, No. 3, 63 pp., 1 fig., 2 tables; February 1947, Ser. II, Vol. 9, No. 4., 41 pp; March, 1947, Ser. II, Vol. 9, No. 5, 34 pp; April, 1947, Ser. II, Vol. 9, No. 6, 36 pp. New York: New York Academy of Sciences, 1947.
- Non-Projective Personality Tests.* HAROLD A. ABRAMSON, KEEVE BRODMAN, HAROLD J. HARRIS, GEORGE C. KILLINGER, BELA MITTELMANN, ZYGMUNT A. PIOTROWSKI, DAVID RAPAPORT, ROY SCHAFFER, MARTIN SCHEERER, DAVID WECHSLER, ARTHUR WEIDER, HAROLD G. WOLFF, EDITH WLADKOWSKY and JOSEPH ZUBIN. New York: New York Academy of Sciences, 147 pp. \$1.75. 1946.
- El Problema de la Circulación de la Sangre. Nuevos Hechos y Nuevas Ideas.* DR. M. BAÑUELOS, Catedrático de la Universidad de Valladolid. 216 pp., 29 figs., 29 tables. Barcelona: Editorial Científico Médica, 1946.
- Les Septicémies à Staphylocoques.* M. BARIÉTY et H. Brocard. 252 pp. Paris: J.-B. Baillière et Fils, 1945.
- Poisons, Their Properties, Chemical Identification, Symptoms, and Emergency Treatments.* VINCENT J. BROOKES, Sgt., New Jersey State Police, and HUBERT N. ALYEA, Associate Professor of Chemistry, Princeton University. 209 pp. New York: D. Van Nostrand Company, 1946.
- Hygiène des Institutions de Plein Air.* H. CAMBÉSSÈDES and J. BOYER. 172 pp., 7 figs., paper. Paris: J.-B. Baillière et Fils, 1946.
- Ambulatory Proctology.* ALFRED J. CANTOR, M.D., Associate Proctologist, Kew Gardens General Hospital, Long Island, New York. 524 pp., 347 drawings, 275 figs. \$8.00. New York: Paul B. Hoeber, Inc., 1946.
- Anais da Faculdade de Medicina da Universidade do Recife Años X-XI Números X-XI.* Professor OSCAR COUTINHO, Director. 171 pp., 35 figs. Recife, Brasil: Imprensa Industrial, 1946 (Distribuição Interna).
- Optical Workshop Principles.* Col. CHARLES DEVE. 306 pp., 120 figs. London: Adam Hilger, Ltd.
- Régimes Alimentaires Usuels de l'Adulte.* GÉRARD DUHAMEL. 170 pp. Paris: J.-B. Baillière et Fils, 1946.
- Conceptions Actuelles du Diabète et son Traitement Hydrominéral.* Professeur A. HANNS. 194 pp., 2 graphs. Paris: J.-B. Baillière et Fils, 1946.
- Physiotherapy.* THOMAS FRANCIS HENNESSEY, M.D., Dean and Director Massachusetts School of Physiotherapy, Boston. 23 pp. \$75. Boston: Bellman Publishing Company, Inc., 1946.
- L'Infirmière Hospitalière, Guide.* Théorique et Pratique de L'École Florence Nightingale de Bordeaux. Tome II, Ed. 4. 645 pp., 18 figs. Paris: J.-B. Baillière et Fils, 1946.
- La Gymnastique Respiratoire et la Gymnastique Orthopédique Chez Soi.* Ed. 3. LOUIS LAMAY. 126 pp., 93 figs. Paris: J.-B. Baillière et Fils, 1946.
- La Psicosis Pelagrosa. Un Análisis Estructural De Los Trastornos Psíquicos.* DR. BARTOLOMÉ LLOPIS, Jefe Clínico del Servicio de Neuropsiquiatría del Hospital Provincial de Madrid. 203 pp. Barcelona: Editorial Científico Médica, 1946.
- Laparatomía Estética en la Mujer.* DR. CARLOS LORCA De la Cruz Española (Madrid), Ex profesor auxiliar de Obstetricia y Ginecología en la Universidad Central; antiguo becario de la Junta Constructora de la Ciudad Universitaria de Madrid; laudado por la Sociedad Ginecología Española. 61 pp., 70 figs. Madrid-Barcelon-Liboa: Editorial Científico Médica, 1946.
- La Formation du Système Nerveux.* RAOUL-MICHEL MAY. 301 pp., 147 figs. Paris: Librairie Gallimard, 1945.

- Les Contagions de la Syphilis.* G. MILIAN. 205 pp., 28 illus. Paris: J.-B. Baillière et Fils, 1946.
- Médecine d'Hier et de Demain.* A. MOLINIER. 119 pp. Paris: J.-B. Baillière et Fils, 1946.
- La Pencilline à la Portée du Practicien.* JEAN MONNIER. 148 pp., 28 figs. Paris: J.-B. Baillière et Fils, 1946.
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NEWS AND NOTICES

MINNESOTA SOCIETY OF CLINICAL PATHOLOGISTS

The Minnesota Society of Clinical Pathologists met at the Mayo Clinic, in Rochester, on Saturday, September 27, and Sunday, September 28, 1947. A Seminar on Hematology by members of the Department of Hematology, under the chairmanship of Dr. Frank Heck, was held at the Foundation House, Saturday afternoon. Papers were presented and discussed, followed by microscopic study of a number of blood and marrow slides. On Sunday morning a scientific session was held at St. Mary's Hospital at which the following papers and demonstrations were presented by members of the Clinic: Addison's Disease, Clinical Aspect, Dr. R. G. Sprague; Biochemical Aspects, Dr. M. H. Powers; Seventeen Kestosteroids, Dr. M. L. Mason; Porphyria, Dr. M. L. Mason; Prothrombin Determination, Dr. F. D. Mann; Surgical Pathology of the Thyroid Gland, Dr. A. C. Broders. On Sunday afternoon, the following were presented: Ovarian Tumors, Dr. M. B. Doekerty; Blood Transfusions, Dr. D. R. Mathieson; and Visit to Blood Bank, Dr. D. R. Mathieson and Dr. T. H. Seldon.

PENNSYLVANIA ASSOCIATION OF CLINICAL PATHOLOGISTS

The Pennsylvania Association of Clinical Pathologists will hold its Spring meeting at Pocono Manor Inn, Pocono Manor, Pennsylvania, April 23-25 under the presidency of Dr. Frederick O. Zillesen. Papers to be presented before the Scientific Session will include, What the Clinical Pathologist Should Know About Atomic Energy by F. W. Sunderman, Discourse on Thyroid Pathology by Alan Graham, Moulage Preparations of Specimens by L. W. Wright, and Work of the State Laboratories by C. P. Brown.

SOCIEDAD CUBANA DE MÉDICOS LABORATORISTAS CLÍNICOS

The first biannual convention of the Sociedad Cubana de Médicos Laboratoristas Clínicos was held at the School of Medicine of the University of Havana, December 9 to 12, 1947. The president, Dr. Antonio Sellek, is an Honorary Member of the American Society of Clinical Pathologists.

IDENTIFICATION OF FUNGI

The attention of readers of the Journal is called to the registry for fungi which is in operation at Duke University School of Medicine. Cultures of fungi may be sent to Dr. N. F. Conant, Department of Bacteriology, School of Medicine, Duke University, Durham, North Carolina, for identification and antigenic study.

PERSONAL

Dr. Bernhard Steinberg, Pathologist and Director of Laboratories at The Toledo Hospital, and Director of Research at the Toledo Hospital Institute of Medical Research, will be relieved of his duties as pathologist and director of laboratories at the hospital so that he may devote his entire time as Director of Research at the Institute. Investigations into the factors which control the production and the distribution of leukocytes are being conducted at the Institute, which is endowed. Problems in glaucoma under a grant from the Snyder Ophthalmic Foundation are also being investigated.

GREEN PASTURES IN PATHOLOGY*

STANLEY P. REIMANN, M.D.

Director of the Research Institute of The Lankenau Hospital and of the Institute for Cancer Research, Philadelphia, Pennsylvania

The American Society of Clinical Pathologists was organized:

- (a) To promote the practice of scientific medicine by a wider application of clinical laboratory methods to the diagnosis of disease;
- (b) to stimulate original research in all branches of clinical laboratory work;
- (c) to establish from time to time, standards for the performance of various laboratory examinations;
- (d) to elevate the scientific and professional status of those specializing in this branch of medicine; and
- (e) to encourage a closer cooperation between the practitioner and the clinical pathologist.

It is a great satisfaction to state that all of these purposes have been carried out to a degree which, I believe, is greater than the founders of the Society had hoped.

The seminars of pathologic anatomy, organized and perpetuated by the Society, have been outstanding, of the greatest assistance to the participants and a model which many local groups have adopted. It is significant that year by year more of the physiologic aspects have been considered. The work in serology, carried out as a cooperative effort on the part of a number of groups, was not only catalyzed, but also worked out in large part by the Society and publicised through it. Studies in hematology have demonstrated the importance of this field, and the Society has had much to do with its development. On the other hand, however much we are inclined to congratulate ourselves on accomplishments, it is, nevertheless, a truism that being satisfied should be the last act of any society, group, or individual. Therefore, what more things can we do, what other fields can we cover, how can we be of wider usefulness to the community?

In several places in the country are groups studying standardization of laboratory methods which is one of the objectives of the Society, and what is equally important and a necessary corollary, *viz.*, how closely do results check in different laboratories using the same methods on the same specimen? A close liaison between these groups and the parent Society is invaluable to the hatching of ideas, their dissemination and action upon them.

The increasing complexities of methods require closer and closer liaison with physiologists, biochemists and physicists. Numerous new methods of analysis were perfected as war-time needs, and many of them have filtered through for the more accurate and more speedy determination of substances of interest in medicine. Full range spectrophotometric analysis is one of them. Electronic

* Presidential address presented at the Twenty-Sixth Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 28, 1947. Received for publication, January 5, 1948.

differentiation of changes in oxidation and reduction is another. Phase contrast lenses are being manufactured, and those who have been privileged to use them have seen things in living tissue better than ever before. As isotopes come into more general use, more and more of us will perforce have to understand the highlights, and details, too. What will we do with long-lived isotopes when we finish a particular experiment? If some of them get loose in the laboratory we may have to dig a deep hole and push the laboratory into it.

Both the choice of tests and interpretation of their results have become more and more specialized. The pathologist, as consultant, has been the subject of numerous papers, and at least one presidential address. There can be no doubt of the increasing need and justification of this phase of his work. In many instances, the laboratory controls almost everything for patients, and sometimes the pathologist is even called in to talk to the relatives. I need not dwell on the implications of this statement.

During the last years, floods of new drugs and new methods of treatment have reached storm proportions. The pathologist, as usual, occupies a key position in their evaluation. Some clinicians "try them out" in haphazard ways, in order perhaps to help a given patient, but certainly not to add to knowledge of their indications, limitations, potencies and toxicities over short or long periods of time. Pathologists can stimulate their clinical colleagues really to study the action of newer remedies and, when such studies are carried out, can be responsible for much data for evaluation. In constructing chemical substances, astounding as it seems, there is at least a beginning of planning compounds and groups for specific purposes. It is, as it were, like architect and builder. One chemist versed in theory lays out a compound on his drawing board, much like the architect draws plans for a building, then turns over his drawings and calculations to another chemist, who, like the builder following the plans of the architect, retires to his laboratory and there proceeds to make the compound. But the theoretical chemist must know what properties are wanted, and here the combined efforts of pathologist and clinician present the needs to him. As examples, consider the methyl group, CH_3 ; if it is labile in the proper compound, no cirrhosis of the liver; if it is absent as a labile group, cirrhosis results; and if the animal (rat) is deprived long enough, tumors appear in a goodly percentage of cases. Pteric acid is being made with variable numbers of glutamic acid groups attached as modifications of folic acid, all with definite desired properties in view. Coleicine derivatives and their relatives are being made to overcome the toxicity of coleicine itself, and yet retain the power of interfering with mitosis. There are many new "anti-histamine" drugs and more coming every day.

In this connection, I cannot help complaining that often I'm not so much concerned with the chemicals themselves and their formulas, although I'm no chemist, as I am by the multiplicity of names given to the same substance by different manufacturers.

We have in our Society a research committee. One of its functions is to define "practical" problems for immediate attack and possible solution. But just as we pathologists can stir our clinical colleagues to investigations, or if not,

at least to place at our disposal properly selected opportunities, so also can we consult with and even help those doing what is called "fundamental" research. I think you will all agree that "art for art's sake" or "science for science's sake" is good for an evening's amusing discussion, but it is too wasteful in time and money just to scout around for problems as a kind of grown-ups' treasure hunt, when there are so many immediate things to do.

There are many leads about us; many examples could be given of lag time between discovery, invention and application. Let us help reduce it. Pathologists are in a position to do this. In any case, research problems should be thought through and organized for effective work; that is, unless we have a genius in our midst. It is gratifying to note that the American Board of Pathology will probably recognize at least six months of full-time research, whether clinical, pathologic, fundamental, or at other levels, as part of the requirements for certification. It should recognize a whole year of the five.

The training of younger men and women in pathology is another of our responsibilities, and it is an important one. Not only must we prepare pathologists technically competent to carry on, but also teach them how to organize laboratory services and to feel the need of no compromise with the ethics of medicine as a whole, and the specialty in particular. A high compliment is paid when pathology is considered so necessary that all of the specialty boards require examinations in it for certification. We help give these examinations; we also spend time preparing residents for them. Ideally, the teaching must be done in such a way that they will remember the principles after the examinations are passed, whether they practice internal medicine, surgery, gynecology, or any of the others. It is another bit of concrete evidence of how widespread our interests and work must be. Among other opportunities is one perhaps not used to full advantage in many instances, *viz.*, liaison with coroners' offices. But, one of our members is an efficient missionary in this direction, and I'll not dwell on medical examiners versus coroners. In any case, the autopsy is still a prime necessity in medicine, even though anatomic changes are not the final word. We examine the entire body; this tells us there is no chance to be narrow specialists; there is every opportunity to be good doctors.

Another committee needs mentioning, and a special plea is made for all to help it. Its task is no less than to try to simplify and standardize nomenclature. Desultory attempts to help physicians understand each other have been made in the past on a number of occasions, but very little has emerged. There are still used at least 33 names for one and the same glandular and connective tissue tumor of the breast. To succeed, it will be necessary for many of us to give up pet names and adopt others, to scrap many obsolete terms, to invent a few new ones. But many advantages are to be gained. At least when we talk to a "lay" audience, as many of us do, for instance about cancer, the listeners will not think we are contradicting each other when one speaks of cancer and another of malignancy or carcinoma. If it is through the use of words that ideas are transmitted, then, indeed, semantics must be an important subject. Judged by the arguments about *words* and not *things*, I dare say that 90 per cent of the time of many committee meetings is spent in exercises in semantics. No doubt the

committee on nomenclature has a hard task ahead of it, but give it support; help it to set up the alternatives and then to choose boldly, for it will never be 100 per cent correct anyway.

Incidentally, or not incidentally, this will also help the enthusiastic, capable and hard-working editor of our Journal, not to mention all of us, if papers submitted to him are written concisely, to the point and with a judicious choice of words. Spend more time on summary and conclusion than on the body of the papers, on the theory that it takes longer to write a short letter than a long one. Send him notes of general interest about persons, procedures and cases. Let us remember that a case, however "interesting" to one of us, is of general interest only if it is made to illustrate some general pathologic or physiologic principle. Sometimes a single case will settle a problem. Over and over again, a pathologic incident has clarified a physiologic question. Finally, in reference to the Journal, a better arrangement has been made with the publishers so that it is now more under our control, and more of its income is available for improvement of the Journal.

Turning to another subject, the past year has seen the organization of the College of American Pathologists. It is gratifying that the national pathological societies have endorsed and are actively assisting in its work. Its purposes are broad, and include education and raising of professional standards, as well as problems in economic fields. It is in our hands to keep its standards high and constantly to raise them. It is another of the all too few organizations in our country which do not lower standards, which do not reduce production and glorify the incompetent, the greedy and the lazy. Among the many activities planned for the College, a number are functioning, an important one being a listing of vacant positions and possible candidates throughout the country. You will hear reports from time to time, clarifying the position of our Society and the division of responsibilities in the educational, technical and economic fields.

In the economic sphere, pathologists are faring better. It would be very satisfying to say that it is due solely to increased appreciation of the pathologists' intrinsic worth. While this is a large part of it, nevertheless, there is a scarcity of well-trained personnel in the field. We are all doing our part to add to the number, but must unite in resisting pressure to hurry. Skills can be acquired more quickly than judgment, and it is judgment in interpretation which is needed. The procedures set in motion are so drastic and the psychologic ramifications so disturbing when the diagnosis of cancer is made from a biopsy specimen, that no one without adequate training should attempt to take the responsibility.

You've heard much of the economics of our specialty, and of medicine in general, during the last years. No need to rehearse the fact that most patients do not meet us personally, as they do clinicians, and in general have a vaguer notion of just what we do. Our brother practitioners can help in this, if they only will.

A peculiarity of our specialty is the serious nature of the work of people we supervise, and for whom, and for whose work, we take full responsibility, *viz.*,

the technologists. They are paid, as we often are, by the abstraction called "the hospital". It is usually a nonprofit corporation owned by no one, and yet owned by everyone who has anything to do with it; indeed, if tax money is accepted, by the public at large. It is usually in debt, for reasons known to many, and its managers are constantly attempting to balance the budget. For some, a good place to make more money, especially by cutting expenses, is the laboratory, since the patients are not so aware of it as they would be if food or immediate service were cut. The College of American Pathologists can help in individual cases by amplifying and fortifying qualifications for laboratory services laid down by the American College of Surgeons and the American Medical Association. The College is our own organization, and its requirements will be those found necessary by all the pathologists in the country. With the same goal in view; *viz.*, the best that medicine has in 1947, 1948 and onward, there is no good reason why basic principles cannot be maintained by both managers and pathologists. We'll certainly do our share, as evidenced in part by what we are doing here.

Still broader ramifications could be discussed, such as the place of medicine in "the changing social order", as the expression has it, or the "future of medicine". How medicine is regarded by any one depends, to a considerable extent, on the individual's political thinking, not to say on his political connections and job. Time is too short for an examination of the philosophy of political systems. But, it can be pointed out that medicine by its very nature is in peculiar intimacy with the daily lives of everyone. Its unprecedented progress in the last fifty years has brought undreamed-of benefits in the field of health, and thus made for less worry and increased happiness and comfort for millions of human beings. No wonder that politicians recognize it as the most potent spearhead for their ambitions, whether they be ward leaders, union leaders, national or international strivers, or what-not. The disguises are numerous and various but their skillfully chosen words always express concern and promises. When we recognize it, and it's not always difficult, why not borrow a leaf from the notebook of any good political exhorter, and use semantics? I take it that none of us wants so-called "socialized medicine" in the ordinary sense of that term. Let's call it "political medicine", and put the stigma on it. We're not opposed in the slightest, to helping the unfortunate, as good doctors have always done, but to help everyone, including those who could help themselves, if they only would, is another question. In fact, we could and would help the public in health matters much more than we do, if only the public would take advantage of what we know and can do. But when a huge majority of our population, perhaps 85 per cent, use physicians only for emergencies, and not for medical advisors as they should, it makes it difficult. Coercion is used in the control of small pox, typhoid fever, and other such diseases, but can it be done in cancer and diabetes? Too many people still use horse-tail tea, or pull off the leaves of a tansy plant from below upward, for an emetic. This is where education is needed, beginning at the lower school levels. We can help in this. Our own education must continue, too. That is why we have this meeting, lest we become dated. Didn't I say in the beginning, we should not be satisfied?

ISOTOPES IN MEDICINE*

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It has been suggested that the greatest benefit to the human race to be derived from the application of nuclear energy may well prove to be the use of isotopes in medicine and in the solution of biochemical problems upon which the science of medicine is based.

More than fifty years have elapsed since the discovery of radioactivity. Its powerful physiologic effects were almost immediately revealed through injury to some of the earliest investigators. The application of this knowledge to the treatment of cancer soon followed, even before physicists had begun to comprehend the nature of the radiations emitted.

Today our knowledge of the structure of atoms and of the forces involved in their transformations is still meager.

We know that an atom consists of a positively charged nucleus surrounded by whirling, negative bodies called electrons. The nucleus is composed of neutrons and protons, the former being electrically neutral while each proton bears a positive charge of one unit, which exactly neutralizes the negative charge on one of the orbital electrons.

The nucleus occupies only about a thousandth of the space of the atom but contains nearly all of the mass, since the weight of each proton and each neutron is approximately 1840 times that of one electron. As atoms are electrically neutral, it follows that there must always be present equal numbers of protons and electrons. Each element has its own characteristic number of these, ranging from one of each in hydrogen, the lightest known element, to uranium, the heaviest known naturally occurring element, with 92 protons and electrons. Hydrogen contains no neutrons, while uranium contains 146, which with its 92 protons, give it a weight of 238.

The number of positive charges on the nucleus, or the number of protons, is known as the atomic number. Hydrogen is designated as ${}^1_1\text{H}$, the subscript on the left referring to the atomic number and the superscript on the right representing the atomic weight. Uranium is written, ${}^{238}_{92}\text{U}$, i.e., 92 protons U 92 protons + 146 neutrons. Obviously, the difference between the atomic weight and the atomic number is equal to the number of neutrons.

Each of the elements lying between hydrogen and uranium in the atomic table possesses its own characteristic number of protons and electrons and therefore has its own specific chemical properties. Addition of a proton to the nucleus, and necessarily also an electron to its orbits, steps the element up one place in the atomic table, while loss of one reduces it to a position next below. This process is known as transmutation, since a different element is formed.

* Received for publication, November 24, 1947.

Alteration in the number of neutrons does not affect the number of electrons, wherefore the chemical properties are unchanged, only the weight is affected. Atoms with identical chemical properties and different atomic weights are called isotopes.

Isotopes are produced by bombarding atoms with various particles derived from other atoms, neutrons, protons, or loose combinations of them, such as alpha particles, which consist of two neutrons and two protons, and hence have a positive charge of two. Deuterons, consisting of one neutron and one proton, and therefore bearing a positive charge of one, also are employed frequently.

The colliding particle may remain in the nucleus of the element bombarded or it may pass through with, or without, knocking out some portion of the nucleus. The newly formed atom may be stable or it may be so unstable as to break down immediately. In still other cases disintegration may occur over a measurable period of time, ranging from less than a minute to billions of years. For each element and set of conditions a characteristic change occurs. Disintegration takes the form of ejection of some definite nuclear constituent, an alpha particle, a beta particle (an electron from a neutron) or a gamma ray, emitted when internal rearrangements take place. This disintegration represents the change by which an unstable atom goes over to a stable form. These disintegrating elements are termed radioactive elements.

Bombardment, by which these isotopes are produced, occurs spontaneously in nature from the action of radioactive elements in ores or may be accomplished in the laboratory by essentially the same process in cyclotrons. Today isotopes are being prepared in chain-reacting uranium piles by neutron bombardment and may be acquired in quantities a thousand to a million times as great as can be procured in a cyclotron. Unstable, or radioactive isotopes of relatively short life, must be produced artificially in these ways but stable isotopes, formed also in nature, slowly accumulate and need only be separated from their more common isotopes.

Methods of separation are based upon the only property in which they differ, weight. A variety of technics are in use, depending mostly upon differential rates of diffusion under set conditions; but, since differences in weight are slight (*e.g.*, methane, $C^{12}H_4$, has a molecular weight of 16 while methane, $C^{13}H_4$, has a molecular weight of 17), separation is slow and in actual practice involves many repetitions of the same process.

Methods of quantitative determination of the isotopes vary according to whether or not they are radioactive. In case they are radioactive, they are measured with a Geiger-Müller counter which registers the number of explosions per second, some instruments even flashing a light and ringing a bell at each count. Stable isotopes are determined for the most part in a mass spectrograph. Here again relative weights (mass) is the critical property.

The accompanying table lists the isotopes which have been most commonly used in biologic work. In the several columns are given the mass (atomic weights), the relative abundance in nature of the stable isotopes and the half-life of the unstable ones. It may be seen that in general, addition of one or two

neutrons to the nucleus permits stability of the isotope, whereas further addition means instability (radioactivity). Loss of one neutron nearly always gives rise to an isotope of high instability, and hence of very short life.

TABLE 1
SOME NATURAL AND RADIOACTIVE ISOTOPES*

ELEMENT	MASS	RELATIVE ABUNDANCE ATOM	HALF-LIFE	ELEMENT	MASS	RELATIVE ABUNDANCE ATOM	HALF-LIFE
		<i>per cent</i>				<i>per cent</i>	
H	1	99.99	—	S	31	—	3.2 sec.
	2	0.01	—		32	95.1	—
	3	—	30 yr.		33	0.7	—
C	10	—	8.8 sec.		34	4.2	—
	11	—	21 min.	Ca	35	—	88 days
	12	98.9	—		36	0.02	—
	13	1.1	—		39	—	4.5 min.
	14	—	10 ⁴ -10 ⁵ yr.		40	96.97	—
N	13	—	9.9 min.		41	—	8.5 days
	14	99.63	—		42	0.64	—
	15	0.37	—		43	0.15	—
	16	—	8 sec.		44	2.06	—
O	15	—	126 sec.		45	—	180 days
	16	99.76	—		46	0.003	—
	17	0.04	—		48	0.19	—
	18	0.20	—		49	—	2.5 hr.
	19	—	31 sec.	Fe	53	—	8.9 min.
Na	21	—	23 sec.		54	6.04	—
	22	—	3 yr.		55	—	4 yr.
	23	100	—		56	91.54	—
	24	—	14.8 hr.		57	2.11	—
P	29	—	4.6 sec.		58	0.28	—
	30	—	2.6 min.		59	—	47 days
	31	100	—	I	124	—	4 days
	32	—	14.3 days		126	—	13 days
					127	100	—
					128	—	25 min.
					130	—	12.6 hr.
					131	—	8 days
					>131	—	2.4 hr.

* Data from R. D. Evans in *Medical Physics*. Chicago: Year Book Publishers, 1944.

Two general types of investigations with isotopes in physiology and medicine are now being pursued. The first is their employment to supplement or supplant x-rays for bombarding tissues. A vast field of research is just being opened up, as it is not as yet certain whether the effects produced by irradiation differ fundamentally according to the type of particle emitted. The effects

vary with the rate of disintegration and the penetrating power of the ejected particles, factors which are characteristic of each element. The amount of the isotope which finds its way to the tissue selected for irradiation varies with the specific element and the compound into which it has been introduced. Thus, it is apparent that to investigate many combinations of these factors adequately, considerable work must be done. But, if the array of possibilities seems bewildering, it must be remembered that the very multiplicity of combinations of these factors opens up just that much more hope for the beneficial application of isotope radiation to therapy.

Phosphorus (P^{32}) has been used most extensively in investigations of cancer treatment. Its half-life, 14.2 days, which means that in 14.2 days one-half of it will have decomposed and in several months all excess radioactivity will have disappeared from the body, is especially suitable. The hardness of its rays is such that it has sufficient penetrating power, about 0.7 cm. of tissue, for it to be useful. Since phosphorus accumulates in rapidly growing tissues and in the bones, where the concentration of phosphorus in exchangeable form is high, it was hoped this radioactive isotope might prove beneficial in the treatment of leukemias in general. In a recent critical survey of the status of P^{32} therapy, Reinhard and co-workers³¹ conclude that "radioactive phosphorus is probably the best therapeutic agent available at the present time for polycythemia vera. Complete hematologic and almost complete symptomatic remissions can be produced with P^{32} in the vast majority of patients, and remission from a single course of treatment may last for from six months to a year or longer."

These authors further conclude that the effect of P^{32} on the clinical course of patients with acute or subacute myelogenous leukemia and those with chronic lymphatic leukemia is about the same as with x-radiation. Acute lymphatic leukemia and monocytic leukemia are not favorably influenced by P^{32} therapy. Further, they conclude that Hodgkin's disease, lymphosarcoma, reticulum cell sarcoma and multiple myeloma respond less favorably to P^{32} than to x-radiation.

The advantages of radiophosphorus administration over roentgen radiation as summarized by Hall and Watkins¹⁷ are: (a) relative ease of administration, (b) absence of radiation sickness and symptoms of toxicity, and (c) simplicity with which the dose can be controlled. The disadvantages are: (a) high cost of radioactive material, (b) bone-marrow injury in case of overdosage, and (c) the possibility of producing a terminal, acute leukemia.

Radiosodium (Na^{24}) has been employed in the treatment of leukemia. Because of the type of its transmissions (β particles and γ rays) and of the generalized distribution it assumes throughout the extracellular and intracellular fluid, its effects resemble those of spray roentgen therapy.

Radioactive strontium (Sr^{89}) has been used to irradiate osteogenic sarcoma and bone metastases from carcinoma of the prostate. Pecher²⁶ employed as high as 10 microcuries, and found no toxic effect and no histologic modification of bone six months after irradiation. The radiation was most concentrated

where the osteoblastic process existed. He concluded²⁵ that on account of the fairly good yield of radioactive strontium that can be produced in the cyclotron, the suitable energy of its rays, and its convenient half-life, it has provided a specific method for irradiation of the skeleton. Treadwell and co-workers,⁴³ on the basis of similar studies, concluded also that the results seemed to justify the therapeutic use of radiostrontium in certain bone tumors.

The employment of isotopes as biologic tracers is the second field of investigation now receiving wide attention. Here again two types of problem may be distinguished. In the first of these, the distribution of the isotope in the body and the rate of its deposition in tissues are used in studies of normal and pathologic growth. Those elements that show predilections for specific tissues are proving especially valuable. For instance, iodine moves rapidly to thyroid tissue, and the rate of its deposition may be followed simply by holding a Geiger-Müller counter near the body. Perlman and his group^{27, 28} found that within two hours from 11 to 17 per cent of labeled iodine fed to rats in tracer doses appeared in the thyroid gland. Analysis showed that from 1.5 to 3 per cent was contained in the thyroxin and as high as 16 per cent was deposited as thyroxin in forty-eight hours.

A further refinement of this method is its extension to the radio-autograph technic,^{3, 18} where sections, obtained at biopsy or after sacrifice of the experimental animal following ingestion of the isotope, are compared microscopically with photographic films against which the section has been held. In this way the position of the isotope within tissues may be observed.

Radioiodine (I^{131} and I^{132} , or a mixture of both) has made possible extensive investigations of the thyroid. It has been found that the rate of deposition is increased in hyper- and decreased in hypo-thyroidism;⁹ but, some cases of elevated and depressed metabolic rates do not show alteration in the rate of iodine deposition and therefore cannot be ascribed to thyroid disturbance. Hence, this test is assuming important diagnostic value.

Two radioisotopes of iron (Fe^{59} and Fe^{55}) have been employed for studying the life history and fate of the red blood cell under normal conditions^{5, 12, 14-16} and in various types of anemias, especially that due to blood loss.¹³ Radioiron was used by Chapin and Ross⁴ to determine the true red cell volume of the blood. They found it to be 8.5 per cent lower than indicated by the centrifuge hematocrit method.

Radioactive sodium (Na^{24}) has been used effectively in studies of mineral metabolism in normal individuals,¹⁰ including fetal-maternal exchange^{8, 29} and in many pathologic states.³⁸ Together with potassium (K^{42}) it has contributed to our knowledge of water balance and ionic exchange, especially exchange through various membrane barriers.^{7, 9, 11, 19, 22, 23, 46}

Since the distribution of strontium in bone follows closely that of calcium,²⁵ this radioelement has been used to study the process of healing after fractures²⁵ and after administration of parathyroid extract⁴⁴ and of pituitary growth hormone.²¹

The second type of tracer study is the employment of isotopes to follow the

metabolic changes of normal body constituents. Throughout the entire history of physiologic research is scattered a series of attempts to find suitable markers by which ingested compounds or fragments derived from them could be identified after passage through the body. Isotopes are the perfect markers. Being chemically indistinguishable from their more common isotopic sisters, they are utilized by cells in the relative concentration in which they and the commonly occurring form are supplied.

Radioactive and stable isotopes are both suitable, and the choice depends upon their relative availabilities and the conditions of the particular experiment to be performed. The high sensitivity of the Geiger-Müller counter, as compared with that of the mass-spectrograph, permits analysis of fractions of a microgram of radioactive compounds as against several milligrams of stable ones. Length of life of unstable isotopes must be taken into consideration, as a half-life of at least several hours is needed for most experiments and frequently a much longer time is required. Hardness or softness of the rays emitted are also an important factor, as it is by these rays that they are determined in the Geiger-Müller counter. C^{14} , Ca^{45} and H^3 are examples of isotopes with radiations so soft that special technics must be employed for their quantitative determination. When a radioactive isotope has been used, the experiment must be completed within about ten times the half-life of the element, varying with the degree of hardness of the ray.

Returning to our table, let us review briefly the uses to which some of the isotopes available for experiments of this type have been put.

Most of the pioneer work of Schoenheimer³⁶ was carried out with H^2 , deuterium. This isotope is relatively easy to get and to measure. At first it was used largely as a marker for the carbon atom to which it was attached. Its usefulness here was limited since hydrogen in carboxyl, amino and other polarized groups or adjacent to a carbonyl group, exchanges rapidly with the hydrogen of the water. Hydrogen attached to a carbon adjacent to a keto-group reacts slowly because of enolization and is said to be semi-labile. Today we no longer need hydrogen for this purpose, since isotopes of carbon are available.

Isotopic hydrogen is still valuable, however, for instance, in studies of hydrogenation and also, since water enters as a component of so many chemical reactions, heavy water is particularly useful in studying rates of synthesis and hydrolysis.

H^3 , tritium, has not yet been widely used, possibly because it has not been readily available. Its radiation is so soft that there is encountered some difficulty in its determination. Since the relative weights of protium, H^1 , to deuterium, H^2 , to tritium, H^3 , are as 1:2:3, the stability of the $C-H^1$, $C-H^2$ and $C-H^3$ bonds vary appreciably. In some cases, rates of reaction are so much slower with tritium, that wide differences in the quantitative ratios of end-products result. Hence, considerable preliminary work must be done before too rigid interpretations of data obtained by the use of tritium can be accepted.

C^{11} is limited in usefulness for metabolic experiments by its short half-life, 20.35 minutes. Its short half-life is compensated for, to some extent, by the

hardness of the radiations it emits. The high specific activity in which it can be obtained, that is, the high ratio of radioactive atoms to the total number of isotope atoms, also tends to compensate, and with careful correction for rate of decay, C^{11} has been a valuable tool for short-term experiments, such as those on photosynthesis. Vennesland and co-workers⁴⁵ used it to determine whether carbon dioxide is utilized by animal tissue (rat's liver) in carbohydrate synthesis. In this experiment, after several practice runs, sodium bicarbonate containing C^{11} was injected into a rat's peritoneal cavity, and after an interval of two and one-half hours, the glycogen was isolated, purified, hydrolyzed, the osazone prepared from the hydrolyzed glycogen and the C^{11} determined all within the five-hour deadline during which the C^{11} retained sufficient activity for determination.

C^{14} has largely replaced C^{11} and is widely used. The long half-life of C^{14} , estimated at approximately 5000 years,^{24, 30} renders it inappropriate for internal administration to human subjects until more precise knowledge has been acquired as to the effects within the organism of long continued bombardment with doses in tracer levels.

O^{18} has been little used, largely because of the difficulty in obtaining it. Although this element in carboxyl, carbonyl and hydroxyl groups exchanges with that in water, the rate is sufficiently slow so that with suitable correction, O^{18} can be used to determine the mechanism of oxidation reactions. Ruben,³⁵ for instance, demonstrated that the oxygen expired by plants during photosynthesis is derived from the water of the medium rather than from the CO_2 , the carbon of which is utilized for carbohydrate synthesis.

Nitrogen has only one available isotope, a stable one, N^{15} . With its use, Schoenheimer³⁶ demonstrated the lability of amino groups, even within the protein molecule, there being a continuous exchange between amino groups of all amino acids excepting lysine, which was not involved in this nitrogen shift. With some amino acids the exchange took place with great rapidity, with others it occurred more slowly. Differences in the rate of amino-group exchanges were also found in different organs, so that degrees of reactivity of organs could be distinguished.

Since phosphorus is a constituent of many essential cell components including phosphoproteins, phospholipids and the various phosphorylating agents associated with the transfer of energy, P^{32} has become one of our most useful isotopes in metabolic tracer work as well as in therapeutics, as mentioned above.

Two isotopes of sulfur, S^{34} , stable, and S^{35} , radioactive, are available for tracer work. Since sulfur is a constituent of all protein, labeled sulfur in addition to labeled carbon may be used as a tracer in studies of protein metabolism.⁴² The relationship of the sulfur-containing amino acids, methionine and cystine, has been partially elucidated in the demonstration that the sulfur of methionine may be converted to cystine sulfur⁴¹ and to taurine sulfur⁴² in the rat. The conversion of methionine sulfur to cystine sulfur has been confirmed⁶ by the use of the stable isotope, S^{34} . The more recent demonstration of sulfur in the vitamins, biotin and thiamin, and in some of the important antibiotics such as

penicillin, together with suggestions that it plays a role in the activity of some of the hormones and of many of the enzyme systems, opens up a field of special interest for the use of the radioisotope. The turnover of thiamin and the fate of its sulfur in a human subject has already been studied² by injecting thiamin synthesized with radioactive sulfur.

The researches of Schoenheimer carried out largely with deuterium and N^{15} , immediately initiated a new era in our concept of metabolic processes by demonstrating that organs and tissues cannot be looked upon as static structural units, but merely parts of one grand chemical system in which distinctions cannot be drawn between structural and metabolic components. He used the term "dynamic equilibrium" to express the idea that both synthetic and degradative reactions were occurring simultaneously with intermediary metabolic fragments forming a so-called "metabolic pool" from which reactions could proceed in many different directions. A demonstration of such a series of phenomena could not have been made without the use of markers like isotopes.

The present-day extension of Schoenheimer's work emphasizes this basic conception. One of the fields that has been intensively investigated with the aid of isotopes is the metabolism of fatty acids. Previously, it could not be demonstrated whether this group of substances was available for reactions in the body other than degradative and oxidative, leading to the release of energy for work. With labeled carbon, largely C^{13} , it has been demonstrated that 2-carbon fragments, derived from the fatty acid molecule, may take part in many diversified processes such as the synthesis of cholesterol,¹ fatty acids,³² succinic acid,³⁷ citric acid,³⁹ glutamic and aspartic acids,³³ glycogen²⁰ and uric acid.⁴⁰

In addition, a new conception of the metabolic relationship of carbohydrate and fat has evolved from the demonstration that in the final steps of oxidation, the carbohydrate fragment acts as a catalyst in the complete oxidation of the 2-carbon residue. Since derivatives of amino acids also enter as components of this catalytic system, we see a tying together of the metabolism of these three great classes of food-stuffs, carbohydrate, protein and fat, a relationship that has been a field of speculation since the earliest metabolic studies in man and in the experimental animal.

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KAHN REACTIONS WITH CARDIOLIPIN ANTIGEN COMPARED WITH KAHN ANTIGEN. II

WITH A NOTE ON A MICROFLOCCULATION PROCEDURE WITH CARDIOLIPIN ANTIGEN*

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It was previously reported² from this laboratory that cardiolipin-lecithin-cholesterol antigen to which a trace of a nonantigenic colloid, such as mastic, is added can be used in the standard Kahn test, side by side with Kahn antigen. At that time it was believed that the addition of the colloid to the cardiolipin antigen was necessary to "mask" visible particles in negative reactions and to increase the size of floccules in positive reactions. Further studies indicated that, by proper adjustment of the cardiolipin, lecithin and cholesterol amounts, it was possible to have an antigen, free from mastic, which behaved in the standard Kahn test essentially like Kahn antigen. In this article, the method of preparation and standardization of mastic-free cardiolipin antigen suspension for the Kahn test, with results obtained in comparison with Kahn antigen, will be presented briefly. It will also be shown that the same antigen suspension employed in the Kahn test may be used in a microfloculation test.

CARDIOLIPIN ANTIGEN FORMULA

In attempting to devise a mastic-free formula for an antigen of cardiolipin, purified lecithin and cholesterol for use in the Kahn test, the first aim was that the antigen with salt solution produce an antigen suspension of dispersible aggregates similar to Kahn antigen suspension. It was believed that a cardiolipin antigen suspension in which the aggregates are dispersible would in all likelihood behave like Kahn antigen suspension in sensitivity and specificity. Since in Kahn antigen the proper concentration of lipids plays an important role in its behavior with salt solution and with serum, it seemed reasonable to believe that, in addition to the use of proper ratios of cardiolipin, lecithin and cholesterol, it would be necessary to employ these reagents in as optimal concentrations as possible. This view led to studies of optimal ratios of these lipids combined with their optimal concentrations. It was observed that the following percentages of cardiolipin, purified lecithin and cholesterol appear to satisfy the requirements of a cardiolipin antigen which behaves like Kahn antigen both in its titration with salt solution and in its reactions with serum:

Purified lecithin	1.0 per cent
Cardiolipin	0.1 per cent
Cholesterol	0.025 per cent

* Received for publication, January 12, 1948.

An outstanding feature of this formula is the relatively high concentrations of cardiolipin and lecithin and the very low concentration of cholesterol. The 25 mg. per cent of cholesterol in cardiolipin antigen matched against the 600 mg. per cent of cholesterol in Kahn antigen, it is believed, might help to bring out selective reactivities of the two antigens in certain situations in syphilis.

It should be added that the extent to which the above formula will be applicable to new lots of lecithin and cardiolipin can only be determined by trial. Experience indicates that this formula will furnish a workable base for the standardization of new lots of cardiolipin antigen.

TABLE 1
TITRATION PICTURES OF CARDIOLIPIN AND KAHN ANTIGENS

CARDIOLIPIN ANTIGEN* SUSPENSIONS: AMOUNTS OF ANTIGEN AND 0.9 PER CENT SALT SOLUTION	DISPERSIBILITY OF ANTIGEN SUSPENSION AGGREGATES: RESULTS IN 3-TUBE TEST, USING SALT SOLUTION INSTEAD OF SERUM	KAHN ANTIGEN* SUSPENSIONS: AMOUNTS OF ANTIGEN AND 0.9 PER CENT SALT SOLUTION
cc.		cc.
1 + 0.8	Cloudy suspension, aggregates not dispersed	1 + 1.1
1 + 0.9†	Opalescent picture, aggregates dispersed	1 + 1.3†
1 + 1.0	Slightly too clear, aggregates dispersed	1 + 1.5
1 + 1.1	Very clear, aggregates dispersed	1 + 1.7
1 + 1.2	Nearly water-clear, aggregates dispersed	1 + 1.9

* Cardiolipin antigen Lot C9; L11-AA; Kahn antigen Lot 107A.

† Antigen titer employed in the Kahn test.

TITRATION PICTURE OF CARDIOLIPIN ANTIGEN

The titration picture of cardiolipin antigen is essentially the same as the titration picture of Kahn antigen. The similarity in the titration pictures of the two antigens is illustrated in Table 1. Both antigen suspensions contain nondispersible aggregates in certain mixtures of antigen and salt solution. Both suspensions begin to show dispersible aggregates as the amount of salt solution is increased, and the suspensions reach water clarity with sufficient increase in salt solution. The titers of the two antigens are not the same. The cardiolipin antigen titer is 1 cc. antigen + 0.9 cc. salt solution while the Kahn antigen titer is 1 cc. antigen + 1.3 cc. salt solution. This reduced amount of salt solution in cardiolipin antigen titers, as compared with Kahn antigen titers, may in part be associated with the reduced amount of cholesterol in the cardiolipin antigen and the relatively large amount in Kahn antigen.

COMPARATIVE KAHN REACTIONS EMPLOYING CARDIOLIPIN AND KAHN ANTIGENS

Table 2 summarizes the comparative Kahn results with cardiolipin and Kahn antigens. Four plus, three plus and two plus readings were interpreted as

positive, while one plus and plus-minus readings were interpreted as doubtful. The serums (1910) used in this study were those sent to the serologic laboratory of the University of Michigan Hospital for routine Kahn tests, and the examinations with the two antigens were made simultaneously. Three hundred of the serums were examined in the Michigan Department of Health laboratory at Lansing, and these were first examined with the Kahn test with Kahn antigen and about two hours later, with the Kahn test with cardiolipin antigen.

It is evident from the table that Kahn reactions with the two antigens show a close degree of parallelism, with 98.2 per cent agreement between the two antigens, 0.3 per cent relative agreement and 1.5 per cent disagreement. The reactions with Kahn antigen are very slightly more sensitive than those with cardiolipin antigen.

TABLE 2

KAHN REACTIONS WITH CARDIOLIPIN ANTIGEN COMPARED WITH KAHN REACTIONS WITH KAHN ANTIGEN IN 1910 ROUTINE EXAMINATIONS

NUMBER OF SERUMS	STANDARD KAHN REACTIONS WITH KAHN ANTIGEN	STANDARD KAHN REACTIONS WITH CARDIOLIPIN ANTIGEN	ANALYSIS OF RESULTS	
			Number	Per cent
163	Positive	Positive	Agreement	
0	Doubtful	Doubtful		
1713	Negative	Negative	1876	98.2
4	Positive	Doubtful	Relative agreement	
1	Doubtful	Positive		
			5	0.3
9	Positive	Negative	Disagreement	
12	Doubtful	Negative		
2	Negative	Positive	29	1.5
6	Negative	Doubtful		

In Table 3 standard Kahn reactions are compared with microflocculation reactions employing cardiolipin antigen. A total of 1740 serums were examined and here also the results are close, with 98.1 per cent agreement, 0.3 per cent relative agreement and 1.6 per cent disagreement.

RELATIONSHIP BETWEEN CARDIOLIPIN AND KAHN ANTIGENS

Cardiolipin antigen represents an outstanding advance in the serology of syphilis, and every effort should be made to employ this antigen, especially in clinical studies and in official evaluation studies in tests for syphilis, so that its value and limitations will become fully established. Cardiolipin has been studied extensively by Pangborn³ who isolated it and who developed methods for the purification of lecithin. The technical application of the antigen in the serology of syphilis has been studied especially by the Maltaners and Brown and by Harris and others. The antigen has not been studied clinically except to a limited degree. Yet, it is already evident that cardiolipin antigen is destined to occupy an important place in the serology of syphilis.

With regard to the relationship between cardiolipin and Kahn antigens in the performance of the standard Kahn test, it is believed that in the present state of limited knowledge of the clinical value of cardiolipin antigen, it would be best to use it side by side with Kahn antigen. In performing the Kahn test, Kahn antigen with its relatively unknown lipid content and cardiolipin antigen with its known and highly purified lipids should, in combination, make a desirable antigenic team. It is naturally assumed that, because cardiolipin antigen is highly purified, it is superior to the older, crude alcoholic extracts. Such an assumption in biology does not, however, always hold true. To mention an example in the field of nutrition, the purification of certain foods may reduce rather than increase their nutrient value by robbing them of essential vitamins.

TABLE 3

STANDARD KAHN REACTIONS COMPARED WITH MICROFLOCCULATION REACTIONS WITH
CARDIOLIPIN ANTIGEN IN 1740 ROUTINE EXAMINATIONS

NUMBER OF SERUMS	STANDARD KAHN REACTIONS WITH KAHN ANTIGEN	MICROFLOCCULATION REACTIONS WITH CARDIOLIPIN ANTIGEN	ANALYSIS OF RESULTS	
			Number	Per cent
42	Positive	Positive	Agreement	
9	Doubtful	Doubtful		
1655	Negative	Negative		
			1706	98.1
2	Positive	Doubtful	Relative agreement	
4	Doubtful	Positive		
			6	0.3
2	Positive	Negative	Disagreement	
1	Doubtful	Negative		
4	Negative	Positive		
21	Negative*	Doubtful		
			28	1.6

* Kahn presumptive test positive or doubtful with 16 serums.

The main weakness of an assumption that a purified antigen is *ipso facto* superior to antigens of crude extract lies in the fact that we do not know the contents of the latter antigens. If, for example, it were definitely known that Kahn antigen consisted of impure cardiolipin, impure lecithin and other impurities, then an antigen consisting of purified cardiolipin and purified lecithin would obviously be superior. But, Kahn antigen may contain various as yet unidentified lipids, perhaps unrelated to cardiolipin, which also are antigenic in syphilis. For example, indications are that Kahn antigen contains lipids which play a protective role in precipitation. We have recently been working with a lipid fraction isolated from Kahn antigen which is far more sensitive than Kahn antigen, thus pointing to protective elements in this antigen. These protective elements are apparently lacking in cardiolipin antigen. If, then, cardiolipin antigen is not a purified counterpart of Kahn antigen, it must be assumed that we are dealing with two distinct antigens. The fact that Kahn antigen contains a relatively large amount of cholesterol, actually 0.6 per cent, and the cardiolipin

antigen described in this article, only 0.025 per cent, is of interest in this connection.

It should be emphasized that the formula for the use of cardiolipin, lecithin and cholesterol described in this article may not be applicable to different lots of these reagents. It is believed, however, that small variations in the formula should be sufficient to bring the final product to the desired degree of sensitivity. A uniform degree of sensitivity is of the utmost importance in an antigen and, in the case of Kahn antigen, uniformity in sensitivity is rigidly adhered to.

With regard to keeping qualities of cardiolipin antigen, based on reports of the New York State Health Department Laboratory, there is every indication that this antigen does not undergo changes on prolonged ageing. Yet, only actual trial with different lots can definitely establish whether or not certain lots might undergo change. In the case of Kahn antigen no changes on ageing were noted during the first decade of its development. Then changes on ageing began to be noted in isolated instances.

TABLE 4

SPECIFICITY AND SENSITIVITY OF KAHN STANDARD TEST EMPLOYING KAHN ANTIGEN AND NEW YORK STATE COMPLEMENT-FIXATION AND FLOCCULATION TESTS EMPLOYING CARDIOLIPIN ANTIGEN IN 1947 EVALUATION STUDY

	SYPHILITIC SERUMS TESTED	SENSITIVITY, PER CENT	NONSYPHILITIC SERUMS TESTED	SPECIFICITY, PER CENT
Kahn Standard*.....	212	81.6	136	100
N. Y. Cardiolipin Flocc.†.....	210	75.0	132	100
N. Y. Cardiolipin Compl. Fix.†.....	213	73.3	135	100

* Performed in the Serology Laboratory, University of Michigan Hospital, Ann Arbor.

† Performed in Laboratory of the New York State Department of Health, Albany.

Changes in Kahn antigen due to ageing can be detected in the changed appearance of negative reactions. To illustrate, an antigen with a titer, let us say, of 1 + 1.2 has aged for two years. On examination of this antigen with positive and negative reacting serums, it is found that the negative reactions appear cloudy instead of opalescent and clear. This finding at once indicates that the old titer of 1 + 1.2 is incorrect for this antigen and that the antigen needs to be retitrated. It might be mentioned that this simple requirement of retitrating certain aged antigens was not adhered to by Webb and associates who reported changes in sensitivity of Kahn and other antigens on ageing.⁴ These workers employed the Kahn antigens at the original titers given on the antigen labels without regard to the fact that certain antigens showed by the appearance of the negative reactions that they should have been retitrated before use in the tests.

Returning to cardiolipin antigen, as it is beginning to be employed in different tests for syphilis, it seems to us that only official competitive evaluation studies can determine readily and impartially (1) which of these tests with cardiolipin antigen is superior to the others and (2) the extent to which this superior test is also superior to any of the tests with alcoholic extract antigens. In 1932, one

of us wrote: "I should like to urge . . . organizations interested in syphilis and its control to arrange a . . . competitive serologic conference similar to those

TABLE 5

THIRTEEN CONSECUTIVE OFFICIAL EVALUATION STUDIES (1937-1947) IN WHICH THE KAHN STANDARD TEST PARTICIPATED IN THE EXAMINATION OF 2293 NON-SYPHILITIC CASES

YEAR OF EVALUATION STUDY	TEST	NUMBER OF NON-SYPHILITIC DONORS	SPECIFICITY, PER CENT	CLINICAL CONDITION
1937	Kahn standard Kahn presumptive	100	100 100	Normal
1938 (1)	Kahn standard Kahn presumptive	96	100 100	Normal
1938 (2)	Kahn standard Kahn presumptive	441	100 99.7	Tuberculosis
1939	Kahn standard Kahn presumptive	114	100 100	Normal
1940	Kahn standard Kahn presumptive	111	100 100	Normal
1941 (1)	Kahn standard Kahn presumptive	130	100 99.6	Normal
1941 (2)	Kahn standard Kahn presumptive	453	100 99.8	Malignancy, fever, etc.
1942	Kahn standard Kahn presumptive	129	100 100	Normal
1943	Kahn standard Kahn presumptive	131	100 100	Normal
1944	Kahn standard Kahn presumptive	161	100 98.5	Normal
1945	Kahn standard Kahn presumptive	153	100 100	Normal
1946	Kahn standard Kahn presumptive	135	100 100	Normal
1947	Kahn standard Kahn presumptive	136	100 99.7	Normal

held at Copenhagen and Montevideo. The author of method or modification A claims it to be superior to methods B, C and D. The author of method or modification B claims it to be superior to A, C and D. The result is that a

worker interested in the serology of syphilis will obtain divergent views depending on whether he seeks his information in certain laboratories in New York, Pennsylvania or Michigan. Not only is there need for a serologic conference in America, but for repeated conferences at least every five years. Such conferences would eliminate unreliable methods and at the same time serve as a stimulus to serologists to further perfect existing methods."

Since 1932 we have become all the more convinced that in a field as complex as the serology of syphilis, with numerous serologic methods and with claims and counter claims as to their superiority, these official, competitive evaluation studies represent the only impartial method of approach in determining the value and limitations of a test. When we indicated above the desirability of employing cardiolipin with Kahn antigen in the Kahn test, we were guided only by results of official evaluation studies. Thus, let us examine the 1947 official evaluation of laboratories on tests for syphilis carried out by the U. S. Public Health Service under the direction of Doctor Mahoney. Flocculation and complement-fixation tests with cardiolipin antigen were performed in the laboratories of the New York State Health Department. Kahn tests were performed in the serologic laboratory of the University of Michigan Hospital. According to the tabulations of results submitted by Doctor Mahoney (Table 4), all the above tests gave 100 per cent specificity. In so far as sensitivity is concerned, the Kahn test gave 81.6 per cent, the cardiolipin flocculation 75 per cent and the cardiolipin complement-fixation 73.3 per cent. The increase in sensitivity of the Kahn test was thus 6.6 and 8.3 per cent, respectively, over the two cardiolipin tests.

The other evaluation results of interest in this connection are summarized in Table 5. This table gives the specificity results of the standard Kahn test in all official evaluation studies from 1937 to 1947. In each of the evaluation studies it gave 100 per cent specificity. The results given by the Kahn presumptive test are also listed in the table by way of comparison and also to indicate that even this more sensitive test gave highly specific results. The 100 per cent specificity record of the standard Kahn does not mean that this test does not give false positives, but, according to the law of averages, it is likely to give fewer false positives than the other methods which from time to time gave false positives in these evaluation studies.

As tests employing cardiolipin antigen participate in official evaluation studies, the tests will not have to depend on the praise of their authors on which to build their reputations. The results in the evaluation studies will praise or blame the tests impartially. Without evaluation studies, it is one opinion against another.

TECHNICAL CONSIDERATIONS ON USE OF CARDIOLIPIN ANTIGEN IN STANDARD KAHN TEST

1. Cardiolipin antigen, standardized for the Kahn test, should be kept in a tightly stoppered bottle at room temperature in the dark. It is presumed that the antigen will not undergo changes in sensitivity and specificity on ageing.

2. In the preparation of the cardiolipin antigen suspension, cardiolipin antigen is mixed (like standard Kahn antigen) with the designated amount of 0.9 per cent sodium chloride solution, in accordance with the titer of the antigen, indicated on the label of the antigen bottle. The measurements should be made precisely, never with 5 or 10 cc. pipets, but with 1 cc. pipets graduated to 0.01 cc. Sodium chloride of the highest purity (reagent quality) should be employed.

3. The cardiolipin antigen suspension should stand ten minutes before it is used, and should be discarded after it has stood for more than thirty minutes.

4. The heating of the serum for thirty minutes at 56 C., the *absolute clarity of the serum and freedom from particles* and the set-up of the 3-tube test are the same as in the Kahn test with standard Kahn antigen. Serums should be employed soon (if possible, within ten minutes) after the heating period. This applies both to the standard and microflocculation technics.

5. After the cardiolipin antigen suspension and serum have been pipetted, the rack of tests is shaken for ten seconds and is permitted to stand for about five minutes. The rack is then placed in the shaking machine and shaken for three minutes at 275–285 oscillations per minute.

6. Following the three minute shaking period, 0.3, 0.1 and 0.1 cc. of 1.2 per cent salt solution are added to tubes 1, 2 and 3, respectively. (In this regard the Kahn test with cardiolipin antigen differs from the Kahn test with Kahn antigen, in which 1.0, 0.5 and 0.5 cc. of 0.9 per cent salt solution are added to tubes 1, 2 and 3, respectively.)

7. The results of the Kahn test with cardiolipin antigen are read immediately after adding the 1.2 per cent salt solution and agitating the rack gently to mix the ingredients. (In the case of the Kahn test with Kahn antigen, a second reading is made after fifteen minutes' standing.) The results of the Kahn test with cardiolipin antigen are averaged, based on the three tube readings.

READING OF KAHN REACTIONS WITH CARDIOLIPIN ANTIGEN

The reading of results of Kahn reactions with cardiolipin antigen is based on the same criteria as the reading of results of Kahn reactions with Kahn antigen. The precipitates in the positive reactions with cardiolipin antigen are not quite as heavy as those with Kahn antigen, but sufficiently heavy to be readily differentiated as 4+, 3+, 2+, 1+ and \pm . The negative reactions with cardiolipin antigen, when highly magnified, will show the presence of finely dispersed particles (very likely antigen particles) to a somewhat greater degree than the negative reactions with Kahn antigen. However, under proper routine conditions of reading results with either antigen, no difficulty will be encountered in differentiating the negatives even from the doubtful reactions, once the reader becomes familiar with the appearance of the negatives.

No standardized method for reading the results could be made applicable to all workers because (1) different workers have different visual capabilities and (2) different laboratories have different reading facilities.

1. *Reading by window light.* When reading Kahn reactions with cardiolipin antigen by holding the rack of tubes in front of a window in a dimmed room,

the negatives will appear opalescent and clear, and the positive and doubtful reactions will appear turbid or cloudy. The four plus reactions showing heavy precipitation can be readily observed without lifting the tubes from the rack. All other tubes showing any degree of turbidity or clouding should be examined individually, lifting each tube several inches above the eye level and slanting it until the fluid is spread into a thin layer. The precipitate will then become readily visible. It is important: (a) For the reader to stand close to the window which provides the source of light for reading; (b) For the other lights in the room to be dimmed, thereby preventing other sources of light from playing on the tube; (c) For the slanted tube to be held several inches *above* the eye level. For optimal reading of results, the window used for reading should be shaded in its upper and lower parts in such a way as to leave a strip of light, the width of which should be determined for each laboratory.

2. *Reading by slit-lamp or by fluorescent light.* Those reading by artificial light will find it desirable to observe first the general appearance of the reactions by holding the rack so in front of the light used as to differentiate readily the opalescent and clear negatives from the cloudy and turbid positives. Then, the positive reactions are examined individually. Tubes showing borderline clouding should, of course, also be examined individually. As in reading by window light, it is important to dim other lights in the vicinity of the reader.

3. *Reading by magnification.* When employing the concave surface of the microscope mirror for reading results, it is also well first to differentiate the negative reactions from those showing some degree of turbidity, as explained above. Those who find the magnification of the microscope mirror insufficient may combine the mirror with a hand lens. *The magnification must be sufficiently low for an individual reader to assure that the negative reactions appear opalescent and easily distinguishable; when the negative reactions begin to suggest the appearance of doubtful reactions, then the magnification is too high for that reader.* As is well known, any colloidal solution, sufficiently magnified, will show particles.

4. *Qualifications for reading Kahn reactions with cardiolipin antigen.* It is assumed that those who will read Kahn reactions with cardiolipin antigen, will have had ample experience in reading Kahn reactions with Kahn antigen. Workers without this experience should learn first to read Kahn reactions with Kahn antigen.

Important: In conducting comparative Kahn tests with Kahn and cardiolipin antigens, it is highly important to carry out the tests *simultaneously* with the two antigens. Thus, (1) Kahn antigen suspension is pipetted into one rack of tubes and cardiolipin antigen into another rack. (2) Serum is added to both racks simultaneously. (3) The racks are shaken for ten seconds at the same time, allowed to stand about five minutes and shaken for three minutes in the same shaking machine. (4) To the rack containing Kahn antigen, 0.9 per cent solution is then added, and to the rack containing cardiolipin antigen, 1.2 per cent salt solution is added, at the same time. (5) The tests with the two antigens should obviously be read as simultaneously as possible to assure true comparisons.

USE OF CARDIOLIPIN ANTIGEN IN KAHN MICROFLOCCULATION TEST

1. The same cardiolipin antigen suspension, described above, employed in the three-tube test is also employed in the microscopic procedure.

2. The cardiolipin antigen suspension should stand ten minutes before it is used and should be discarded after it has stood for more than thirty minutes.

3. Absolute clarity of the serum and freedom from particles, as well as clean slides, are essential.

4. On a paraffin-ringed slide are deposited 0.05 cc. amounts of the serums, previously heated for thirty minutes at 56 C.

5. By means of a tuberculin syringe with a 23-gauge needle, the antigen suspension is first mixed well by drawing it up several times. The syringe is then held *vertically* over the serum, and a drop of the suspension is permitted to fall in the center. The serum:antigen suspension ratio is then approximately 8:1.

6. The slide is vigorously agitated in a circular motion (150–160 times per minute) for four minutes, and the results are read under low power magnification of approximately $\times 50$.

7. Reading of results: (a) *Negative reactions* in the microscopic field show evenly distributed, very small, non-aggregated particles. (b) *Doubtful* (\pm) *reactions* show a microscopic field crowded with numerous, small aggregates or clumps. (c) *Doubtful* (+) *reactions* show slightly larger aggregates, somewhat less crowded. (d) *Positive* (++) *reactions* show larger aggregates with a corresponding clearing of the field. (e) *Positive* (+++) *reactions* show relatively large aggregates scattered through the clear field. (f) *Positive* (++++) *reactions* show but few large clumps in a clear field.

STUDIES IN PROGRESS

Experimental studies are in progress aimed at increasing the sensitivity of tests with cardiolipin antigen. When considering the 166 cardiolipin positive reactions against the 176 Kahn positive reactions in Table 2, it is evident that the results with cardiolipin antigen are somewhat less sensitive. Preliminary serum quantitative and spinal fluid studies with cardiolipin antigen indicate general agreement with Kahn antigen results but also of somewhat lower sensitivity. Another aspect under investigation is whether optimal precipitation zones given by Kahn antigen with different syphilitic serums¹ are also given by cardiolipin antigen. In so far as specificity is concerned, indications are that the results with cardiolipin antigen are somewhat more specific. To what extent this increased specificity is the result of the reduced sensitivity, is yet to be determined.

SUMMARY

The application of cardiolipin antigen to the standard Kahn test and to a microflocculation procedure is described. It was observed that appropriate ratios of cardiolipin, purified lecithin and cholesterol, combined with optimal concentration of these reagents, result in an antigen which in its titration with

salt solution and in its reactions with serum behaves like standard Kahn antigen. This cardiolipin antigen was found to give sensitivity and specificity results with serum in a slightly modified standard Kahn technic and in a microflocculation technic that broadly parallel the results with standard Kahn antigen. It is believed that, until the value and limitations of cardiolipin antigen are fully established, this antigen should be used side by side with Kahn antigen.

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ISOIMMUNIZATION WITH THE A AND B FACTORS AND ITS RELATION TO HEMOLYTIC DISEASE OF THE NEWBORN*

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Soon after Philip Levine's epochal discovery of the role which the Rh factor plays in the pathogenesis of erythroblastosis fetalis, it was contended that antigens other than Rh also may be capable of inducing isoimmunization and that such isoimmunization may be responsible for some instances of erythroblastosis fetalis in which the Rh or Hr factors played no part in the pathogenesis of the disease. The following pertinent observations to date support this contention:

1. The demonstration by Hirsfeld and Zborowski,⁴ in 1926, and independently by Polayes *et al.*,⁹ in 1927, that isohemagglutinins traverse the placental barrier from mother to fetus.
2. The demonstration of the antigenic properties of human blood by Jonsson⁵ in 1936.
3. The identification of an immune atypical intragroup hemolysin which was responsible for a transfusion reaction post partum in a group A mother delivered of a group B anemic infant, reported by Levine and Polayes⁷ in 1941.
4. The observation by Levine,⁸ in 1943, that a definite relationship exists between A-B-O isoimmunization and spontaneous abortion.
5. The observations and case reports by Polayes,¹⁰ Boorman *et al.*,² Kelsall,⁶ Halbrecht,³ Aubert,¹ Wiener,^{14, 17} and others, between 1942 and 1946, all indicating the possibility that the A and B antigens may induce isoimmunization in Rh-positive women, resulting in neonatal conditions very similar to, if not identical with, erythroblastosis fetalis.
6. The statistical fact that most instances of erythroblastosis fetalis, occurring in infants born of Rh-positive mothers, occur with heterospecific pregnancies, noted by Levine,⁸ in 1943, and by Halbrecht,³ in 1944. The latter analyzed 10,000 births among which he found 60 cases of icterus which simulated mild erythroblastosis and which he called "icterus neonatorum precox". He made the striking observation that in 95 per cent of these cases, the blood of the mother was incompatible with that of the offspring (heterospecific pregnancy). In marked contrast to this, in a comparable series of 2000 pregnancies which terminated with normal babies, he found only 26.5 per cent incompatibility between mother and baby.
7. Finally, the observation by Polayes^{11, 12} and others that mothers (Rh-positive or negative), whose heterospecific pregnancies result in erythroblastotic

* Aided by a grant from the United Hospital Fund of Greater New York. Presented at the Twenty-Sixth Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 26, 1947. Received for publication, October 25, 1947.

infants, show much higher isohemagglutinin titers than do mothers of normal babies. In fact, the high titer of the isohemagglutinins sometimes serves as an aid in prenatal blood group determination. The results of a study of such a series have been discussed in a previous publication.¹²

In 1945, one of us¹¹ called attention to a collection of 6 cases of erythroblastosis fetalis in which the Rh and Hr antigens were excluded as possible immunizing antigens, but in which the mothers, all of group O, showed high anti-A agglutinin titers in their serums. In 1 case which terminated fatally, postmortem examination showed the classic anatomic changes characteristic of erythroblastosis fetalis. The brain presented kernicterus, which, as I have previously reported,¹⁰ is almost a pathognomonic finding in hemolytic disease of the newborn. The other 5 patients suffered from a relatively mild form of the disease, although the anemia persisted even after the earlier jaundice had disappeared. The babies responded well to homologous blood transfusions.

TABLE 1
CONTROL STUDY OF ANTI-A ANTIBODY TITERS OF SERUMS OF 100 WOMEN

TITER	PREMARITAL	PRENATAL	POSTPARTUM
0-100	10	6	3
100-200	2	12	2
200-300	0	0	0
300-500	5	12	6
500-700	6	8	6
1M-2M	3	6	2
2M-5M	0	0	4
5M-10M	1	2	1
10M-20M	0	2	0
20M-30M	0	0	1
Number of women.....	27	48	25

In conjunction with the clinical and pathologic study of these cases, simple experiments were carried out in which the bloods of approximately 50 consecutive group O nulliparas were examined for anti-A agglutinin titer. The average titer was found to be 1:58 (range 1:20 to 1:100). In another comparable series of 50 group O mothers of normal group A children, the average titer was found to be 1:215 (range 1:120 to 1:300). These values were in marked contrast to the high titers of 1:710 (range 1:700 to 1:750), obtained in the six group O, Rh-positive mothers of erythroblastotic group A infants. This difference in titer (Fig. 1) was considered to be significant of isoimmunization of the O mother by the A infant, by a mechanism similar to Rh isoimmunization (Fig. 2). In summarizing that report, it was stated, that although more statistical data would be required to arrive at any conclusions, it was felt that (a) isoimmunization by the A and B agglutinogens may occur, and that (b) erythroblastosis fetalis may result from it, by a mechanism similar to that already established for the Rh factor.

Since then, others have reported similar cases with similar conclusions, as indicated above in the enumerated list of pertinent observations supporting our original contention. The difference in the isohemagglutinin titers which was observed in the various groups (nulliparas, multiparas and heterospecific pregnancies which terminated in erythroblastotic infants) tested in the earlier series, however, indicated the advisability of repeating the same study on a larger number of individuals. Accordingly, the bloods of another series of controls, consisting of 250 women and 9 children, were studied. The results of these studies are given in Tables 1 and 2. The first 100 routine blood specimens of this series were grouped in three categories, namely premarital, prenatal and post partum. A more sensitive titration technic (see below) was employed than the one used in the earlier series, in which the average titer was approximately one-half that obtained by the new technic. Thus, with the new method, the 6 cases of erythroblastosis previously reported would probably have shown an average titer of 1:1420 or more, instead of 1:710.

TABLE 2
AGGLUTININ TITERS OF VARIOUS GROUPS OF INDIVIDUALS

GROUP	NUMBER OF CASES	AVERAGE TITER	
		Anti-A	Anti-B
Children.....	9	1:163	1:240
Nulliparas (nulligravidas).....	12	1:640	1:178
Primiparas			
Compatible.....	129	1:272	1:241
Heterospecific.....	9†	1:364	1:810

† In 7 of these 9 cases, the babies possessed the B antigen, but their mothers did not.

It will be noted from Table 1 that 85 per cent of the premarital group of 27 cases fell within a range of titer of from 1:40 to 1:640, with an average value of about 1:350. About 90 per cent of the prenatal group of 48 cases fell within a range of titer of from 1:40 to 1:2000, with an average titer of about 1:1280. When this group was broken down, the women with compatible pregnancies showed an average titer not exceeding 1:500, no one exceeding 1:1280, while in the heterospecific group, which included 2 women with titers of 1:20,000, the majority showed a range of titer of from 1:500 to 1:5000. In the postpartum group of 25 cases, 72 per cent fell within a range of titer of from 1:300 to 1:2560, with an average titer of about 1:1280. In evaluating the findings, it should be borne in mind that probably none of these three groups was pure. Thus, among the premarital individuals, upon inquiry, it was learned confidentially that some of them had already been pregnant. Similarly, the prenatal group included primiparas as well as multiparas, with compatible, as well as heterospecific pregnancies. The postpartum group also was somewhat heterogeneous in that it included heterospecific as well as compatible pregnancies. For these reasons it was necessary to reclassify these two groups into compatible and heterospecific

pregnancies as given above. Despite the heterology within the various groups, however, the trend of the titration values seemed to indicate that gestation is associated with an increase in the mother's isohemagglutinin titer, probably as a

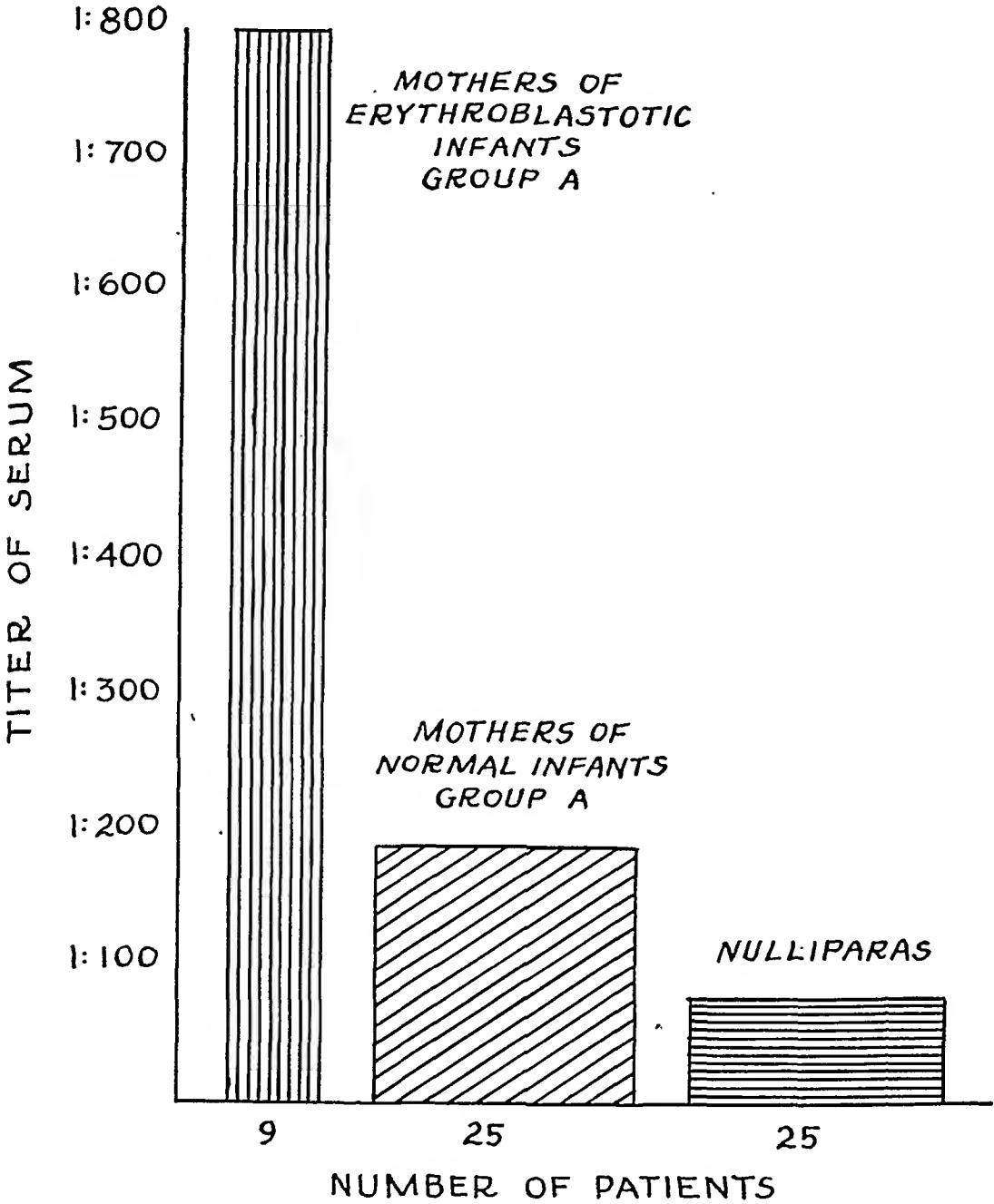
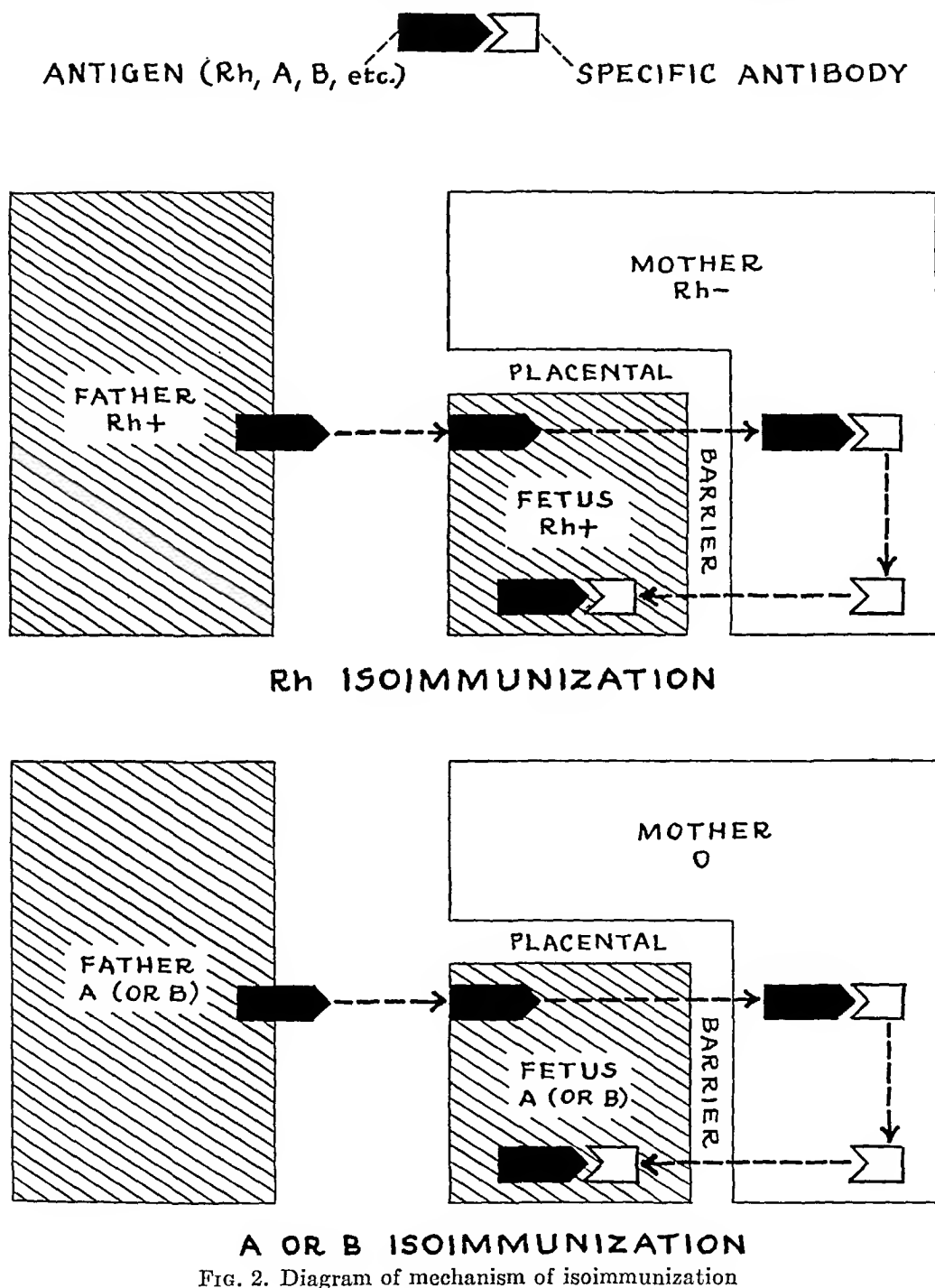


Fig. 1. Serum anti-A agglutinin titration of group O women

result of A-B-O isoimmunization. Figure 3 shows this trend graphically. The trend becomes most marked in the pure group of heterospecific pregnancies mentioned above. The "glutinin" values noted in the graph of Figure 3 were determined under conditions for detecting blocking antibodies.¹⁵ The exceptionally high titer of 1:5000 agglutinin and 1:1280 "glutinin" in one individual in the

premarital group could not be satisfactorily explained. There was, however, some reason to suspect previous pregnancy in this instance. It should be noted



that the comparatively high titer in this one person served to elevate the average titer of the entire premarital group considerably above that which it would have

been ordinarily. Similarly, in the prenatal and postpartum groups there were one or two cases of exceptionally high titer in each, elevating considerably the average titer of their respective groups. As will be observed from a study of the graph, however, the general trend compares favorably with the titers found on the first series of 100 cases, shown in Figure 1. Here, as in the previous study, the pregnant women showed a distinctly higher isohemagglutinin titer than did the non-pregnant women.

The remaining 159 cases were divided into more homogeneous groups. They consisted of 9 children and 12 nulliparas (both serving as controls), and 138 primiparas of which 9 had heterospecific pregnancies. Agglutinin titrations

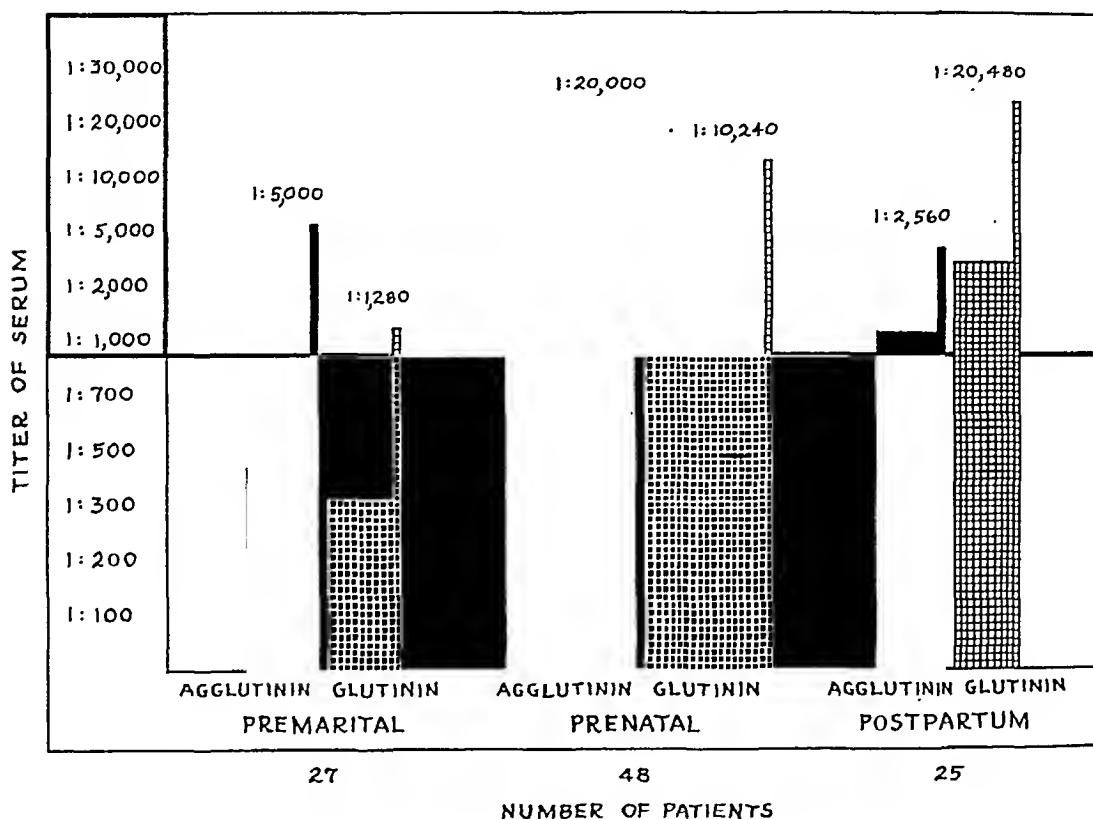


FIG. 3. Diagram showing tendency to higher antibody titers in pregnancy (see Table 1)

only were performed on the bloods of this series.^{16*} The average titer for each group is given in Table 2. It will be noted that this series is made up predominantly of primiparas, multiparas having been entirely excluded. The purpose was to determine to what extent, if any, A-B-O isoimmunization occurs with the first pregnancy. As will be observed from a study of Table 2, the only

* A. S. Wiener was first to point out that it is not merely the *titer* but the *quality* of the antibody which determines whether the baby will be affected. He cites 2 cases "which had the highest agglutinin titer to date, yet the babies in the incompatible blood group did not turn a hair". Dr. Wiener expects to discuss this phase of the subject in a future publication. With these facts in mind, it seems highly desirable to perform conglutination tests on all subsequent cases of this type to be studied in the future.

primiparas who show any significant rise in titer are those with heterospecific pregnancy. As previously stated, in a mixed group of heterospecific pregnancies, including multiparas who presumably had been repeatedly exposed to isoimmunization, the average titer was found to be much higher than the values shown here.

The relatively high titers in this group of nulligravid women probably can be explained by the fact that the group consisted of a relatively small number of individuals, a few of whom happened to have had higher titers than usual. A possible explanation for the high titers in this group is that it was composed entirely of nurses who had recently received prophylactic immunizations. A larger series of nulliparas would undoubtedly show a much lower average titer, as already demonstrated in the two previous series of titrations in which the average titer for nulliparas was found to be about 1:300.

During the course of these studies, 4 cases were encountered, simulating erythroblastosis fetalis, in which the Rh and Hr factors had been excluded as the immunizing antigens. A brief resumé of each of these cases follows.

REPORT OF CASES

Case 1

C. J. (C. H. #128227), a Negro full term male, was born May 19, 1946. The mother, a group O, Rh-positive Hr-positive, para 5, grvida 9, was a treated luetic who had a negative Wassermann test, and had received a full course of treatment before this pregnancy. The baby was delivered partly asphyxiated with a low foreeps laceration over the left zygoma. Its blood group was determined to be B, Rh-positive. Skin and sclerae were intensely icteric at birth and the amniotic fluid was yellow. The blood Wassermann and Kline tests were negative. The jaundice deepened progressively and was severe on the fifth day. The blood count on that day showed 3,750,000 red blood cells per cu. mm., hemoglobin 81 per cent and 90 nucleated red blood cells per 100 leukocytes. There were 12,000 white blood cells, 2 myelocytes, 3 metamyelocytes, 13 stab forms, 33 polymorphonuclears, 38 lymphocytes, 10 monocytes and 1 eosinophil. The prothrombin time was thirtyfive seconds (normal control, thirty seconds), bleeding time one minute and coagulation time two minutes. The baby was given two transfusions, each of 80 cc. group B, Rh-positive blood in two days, to combat declining blood values. Three days later another transfusion of 90 cc. of group B, Rh-negative blood was given with very little improvement. The resident physieian had hoped that the negative blood might be more beneficial. At this time the red blood cell count was 3.5 million per eu. mm. with 3 per cent normoblasts and the hemoglobin was 76 per cent. Although the child appeared worse at first, the jaundice later began to subside gradually. The liver and spleen, however, were palpated 2 fingerbreadths below the right costal margin. During the following ten days the infant gradually improved and was then discharged with a diagnosis of icterus gravis neonatorum. The baby remained jaundiced for about one month after discharge from the hospital.

Examination of the mother's serum showed an anti-A and anti-B agglutinin* titer of 1:2560, an anti-A "glutinin" of 1:2560 and an anti-B "glutinin" titer

* Agglutinins may be defined as bivalent specific antibodies found in the serum of individuals sensitized to any antigen, in this instance the A or B factor. "Glutinins" are univalent specific antibodies, such as blocking antibodies, found in individuals sensitized to any antigen, in this instance, the A or B factor.

of 1:20,480. The nonspecific increase in the anti-A titer was difficult to explain except on a basis of possible previous immunizations with the A antigen. The exceedingly high anti-B "glutinin" titer, however, was considered indicative of B immunization.

Case 2

(Methodist Hospital, Case #32983, courtesy of Dr. B. A. G. Weisl and Dr W. F. Watton).

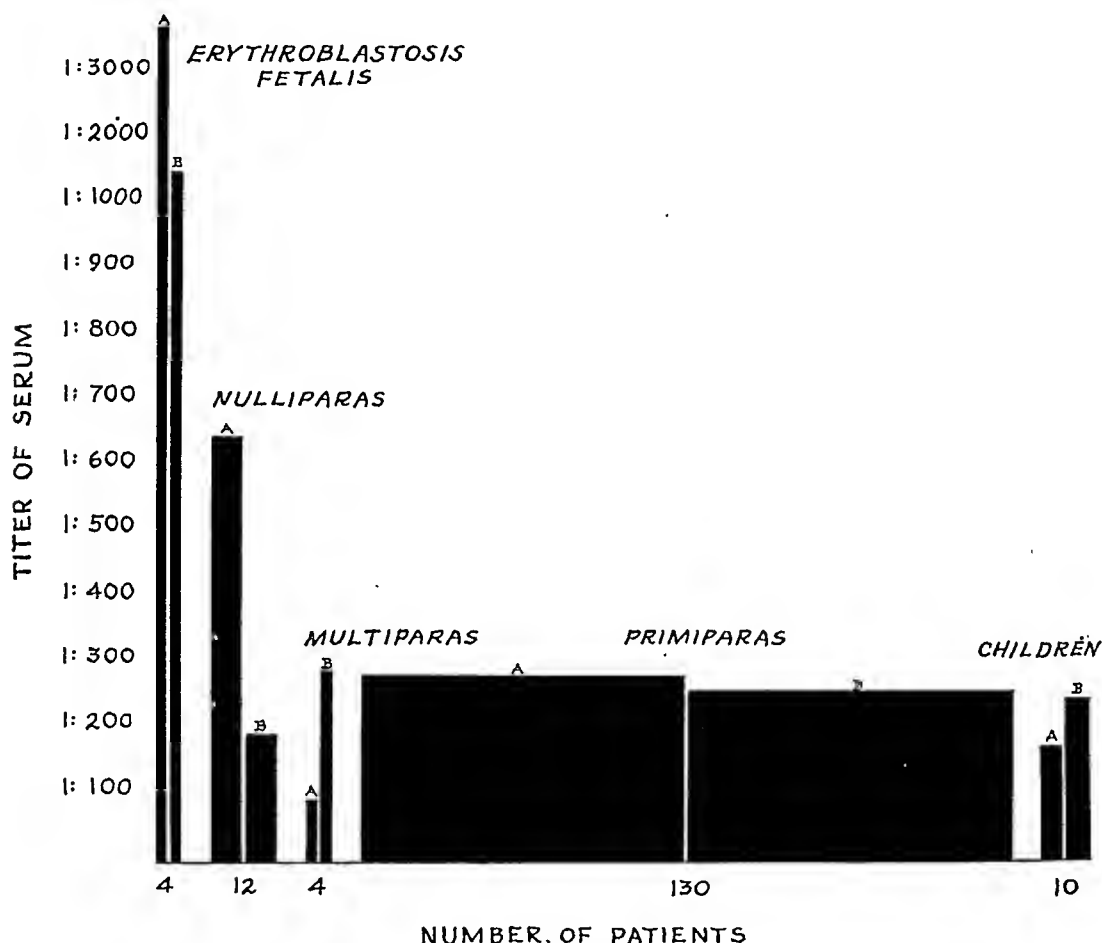


FIG. 4. Graph showing homogeneous group distribution according to titer in 160 cases. Note contrast in the average titer between erythroblastosis group and the others.

S., a full term, white male was delivered by breech presentation on October 13, 1946, from a nullipara, gravida 1, group O, Rh, Hr mother. The baby developed jaundice within the first twenty-four hours. The blood count on the next day showed the following: erythrocytes 4.1 million per cu. mm., 10 per cent normoblasts, hemoglobin 16 Gm., leukocytes 23,000 per cu. mm., with 75 per cent polymorphonuclears, 21 per cent lymphocytes and 4 per cent monocytes. A transfusion of about 75 cc. of blood was given on the second day and again on the ninth day. Although the normoblasts remained at 10 per cent for several days, the other blood values remained fairly normal. The jaundice persisted for nearly one week, however, and was accompanied by a definite hepatosplenomegaly.

The baby's blood was determined to be A, Rh₂ Hr. There were no Rh immune bodies demonstrable in the mother's serum, but the anti-A agglutinin titer

was 1:2560 and the anti-B agglutinin, 1:1280. The child made an uneventful recovery and was discharged as cured on November 6, 1946, with a clinical diagnosis of erythroblastosis fetalis.

Case 3

C. (C. H. #135330), a full term Negro female, was delivered by cesarean section on April 10, 1947. The mother was a para 3, gravida 3, with a negative serologic test for syphilis. Her blood group was determined to be O, Rh-positive Hr-positive. The baby presented an hydrocephalus, requiring aspiration of 150 cc. of cerebrospinal fluid from the ventricles in order to enable delivery of the head. The scalp was lacerated during delivery. At birth, the child had convulsions and there was noted a lower facial paralysis, fixed pupils and petechiae of the skin and conjunctivae. Jaundice appeared within forty-eight hours. At this time, the erythrocyte count was 5 million per cu. mm., with 102 per cent hemoglobin and 42 per cent normoblasts. The leukocyte count was 7300 with 13 per cent polymorphonuclears, 80 per cent lymphocytes, 2 per cent monocytes and 5 per cent eosinophils.

The child's blood group was found to be A, Rh-positive Hr-positive. The jaundice persisted and the infant failed to recover and died on the sixth day. The clinical diagnosis was hydrocephalus with possible erythroblastosis fetalis.

Postmortem examination of the baby showed hydrocephalus, icterus, peritoneal and pleural effusions, pulmonary atelectasis and hepatosplenomegaly with marked erythropoiesis.

The mother's serum showed an anti-A agglutinin titer of 1:5120 and an anti-B agglutinin titer of 1:1280.

Case 4

(Caledonia Hospital. #55928, courtesy of Dr. E. R. Marino). E. C., a white, full term, female, was born May 3, 1947. The mother, a nullipara, gravida 3, had had two previous abortions, one in 1944 and another in 1945. Her blood group was determined to be O, Rh-negative Hr'-positive with no Rh antibodies in her serum. Her blood Wassermann test was negative. The baby developed jaundice in less than twenty-four hours after birth, at which time the blood count was as follows: hemoglobin, 112 per cent; erythrocytes, 4 million per cu. mm. with 31 per cent normoblasts; leukocytes, 16,500 per cu. mm., with 6 per cent immature granulocytes, 55 per cent polymorphonuclears, 35 per cent lymphocytes, 2 per cent eosinophils, 1 per cent basophils and 1 per cent monocytes. The next day the hemoglobin dropped to 98 per cent and the erythrocyte count to 3 million per cu. mm. The spleen was palpable and jaundice progressed. After the baby's blood group was found to be A, Rh-negative, Hr'-positive (the same as the mother's), 125 cc. of A, Rh-negative blood was given on the second day of life. The improvement in the hemic component was only moderate and the jaundice progressed to a severe degree on the fourth day as the child became more listless. Daily transfusions of 100 cc. each were then given for the next few days, with gradual improvement. On May 14, 1947, the jaundice began to diminish, and the child began to show definite clinical improvement, which continued until May 22, when the baby was discharged with a clinical diagnosis of erythroblastosis fetalis (icterus gravis neonatorum).

Examination of the mother's serum showed an anti-A agglutinin titer of 1:5120 and an anti-B agglutinin titer of 1:640.

SUMMARY

The titer of the anti-A and anti-B isohemagglutinins was determined in 259 individuals, including children, nulliparas, primiparas and multiparas, some of

the women having compatible, and others, heterospecific pregnancies. Among those with heterospecific pregnancies, were included four mothers of infants with conditions closely simulating erythroblastosis fetalis, from which the Rh and Hr factors had been excluded as the immunizing antigens. The results seem to indicate that gestation, particularly when it is heterospecific, is associated with an increase in the isohemagglutinin titer, as compared to the titers found in children and in nulligravidas and primiparas with compatible pregnancies. The primiparas who showed any significant rise in the isohemagglutinin titers were those with heterospecific pregnancy. In general, multiparas as a group showed elevated titers, probably due to the fact that as a group, they were exposed to the possibility of repeated isoimmunizations through heterospecific gestation. The highest titers, however, were obtained in those women who had given birth to offspring with conditions very closely simulating and often, clinically and anatomically, indistinguishable from hemolytic disease of the newborn. The average titer in these cases was very much higher than in the other heterospecific pregnancies which resulted in normal babies.

The above findings confirm the earlier observations that the A and B antigens are capable of inducing isoimmunization in pregnancy and that neonatal disease, indistinguishable from erythroblastosis fetalis, may result therefrom. If the term hemolytic disease of the newborn is not acceptable for this group of babies, some other name will have to be given to it. The constant association of such high isohemagglutinin titers in the mothers of these infants identifies them as a distinct group, and the antibody titer must have a particular and related significance, just as the Rh antibodies have in hemolytic disease of the newborn. The clinician is often unable to distinguish the condition of these babies from that resulting from Rh immunization. Often these babies are as gravely ill as the Rh babies, and with the same signs and symptoms. In some instances, the condition is also fatal and on postmortem examination these infants reveal the same anatomic changes which the fatal Rh cases show. The response to treatment is also similar to that shown by the Rh babies.

The arbitrary and complete separation of erythroblastosis fetalis on the basis of immunization by one antigen only (Rh) has already proved to be fallacious. The role which Hr factor has been shown to play in the pathogenesis of erythroblastosis, is a case in point. Still other antigens may yet be uncovered which can play the same role. While the Rh factor is admittedly, by far, the most frequent antigen involved in the pathogenesis of erythroblastosis fetalis, the results of the above investigations indict the A and B antigens as additional offenders. They may well be the immunizing antigens in the above mentioned group of neonatal diseases which so closely simulate hemolytic disease of the newborn due to Rh immunization, as to make it impossible to differentiate them from that group. Therefore, until a more suitable name is found for this group, it seems advisable to consider it a variety of erythroblastosis fetalis.

CONCLUSIONS

1. The antigens A and B are capable of inducing isoimmunization in pregnancy. This is in part evidenced by the fact that the highest isohemagglutinin

titers are found in heterospecific pregnancy, as determined from serum titrations on 350 individuals

2. Women with heterospecific pregnancy, who give birth to offspring with neonatal disease simulating erythroblastosis fetalis due to Rh immunization, usually show very high anti-A and/or anti-B isohemagglutinin titers.

3. Isoimmunization with the A and B factors in pregnancy may result in a variety of erythroblastosis fetalis.

TECHNIC OF SERUM TITRATION FOR ANTI-A AND ANTI-B ANTIBODIES

A. Saline Suspension Method

Make several dilutions of the serum to be tested, the dilution ranging from 1:10 to 1:5120, as follows.

Using a 1 cc. serologic pipet graduated in hundredths, pipet exactly 0.9 cc. of physiologic saline into the first of 10 test tubes ($4 \times \frac{1}{2}$ inches), set them in a rack and number them consecutively from 1 to 10. Using a 5 cc. serologic pipet graduated in tenths, place 0.5 cc. saline into each of the remaining 9 tubes. Draw 0.2 cc. of the serum into a serologic pipet graduated in thousandths. Remove all excess serum adhering to the outside of the pipet by carefully wiping it with a clean cloth. This step is most essential and should be carried out meticulously. Expel 0.1 cc. (only) of the serum from the pipet into tube 1, being careful that the serum enters the saline below the surface level of the latter. With this technic, the 0.1 cc. serum can be delivered into the test tube more accurately than by drawing only the desired 0.1 cc. into the pipet. Be sure not to use the same pipet again in this titration. With a 1 cc. serologic pipet carefully transfer exactly 0.5 cc. from tube 1 to tube 2, again carefully wiping the outside of the pipet before making the transfer. Using a fresh 1 cc. pipet for each dilution, repeat the above step, transferring 0.5 cc. from tube 2 to tube 3, and so on up to and including tube 10. The serial dilutions of the serum will then be 1:10, 1:20, 1:40, 1:80, 1:160, 1:320, 1:640, 1:1280, 1:2560 and 1:5120 in tubes 1 to 10, respectively.

The "test" rack is now set up. Ten tubes, each $4 \times \frac{1}{2}$ inches, are used again for this part of the test. In titrating group O individuals, two sets of 10 tubes in each will be necessary, one for the anti-A and the other for the anti-B antibodies. For group A and B individuals, only one set of tubes is required in the "test" rack. Transfer from serum dilution tube 10, exactly 0.15 cc. into tube (or tubes) 10 of the "test" rack. Blow the serum out of the first pipet as completely as possible, and with the same pipet transfer exactly 0.15 cc. of diluted serum from tube 9 into the "test" tube or tubes 9 as before. Do the same with each succeeding tube using the same pipet throughout. (The 0.15 cc. volume is more exact than 2 drops.)

Fill a 1 cc. serologic pipet with a 5 per cent red blood cell suspension in saline. (For a group O individual, A and B cell suspensions are, of course, necessary.) Permit only one drop of the cell suspension to fall into each of the "test" rack tubes 1 to 10, the A cells for determining the anti-A titer and the B cells for the anti-B titer. Shake the racks and incubate for fifteen minutes in a water bath at 37 C. Resuspend the cells by shaking again and centrifuge at low speed for one minute. Shake tubes gently before reading. The readings are made by examination of a drop of the content of each tube on a slide, under the microscope. The presence of definite clumps, however small, is reported as positive agglutination. The highest dilution showing positive agglutination is the titer reading.

B. Conglutination Technic

The procedure here is exactly the same, except that the cell suspensions and serum dilutions are made with AB serum as diluent instead of saline.

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CLINICOPATHOLOGIC CONFERENCE*

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CLINICAL DATA

Dr. F. F. Rogers. F. Y., a 59 year old Mexican male, was admitted to the John Sealy Hospital at 2 A.M. on September 30, 1947 with severe abdominal pain. The patient stated that on the preceding day he had worked and was well. He ate a large supper and, approximately two hours later, developed an acute, severe, boring pain in the upper portion of his abdomen, with radiation of the pain into the left side of his back. He vomited several times before being admitted to the hospital.

On admission, the upper portion of the abdomen was markedly rigid and no intestinal sounds were audible. Examination revealed slight enlargement of the heart and a systolic aortic murmur, numerous rales over both lung bases and pretibial edema (grade I). No other significant physical findings were elicited. The blood pressure was 122 mm. Hg., systolic and 68 mm. Hg., diastolic; the temperature (rectal) was 99.2 F., the respiratory rate 18 to 22 per minute and the pulse rate 78 per minute. The leukocyte count was 14,300 with 96 per cent polymorphonuclear leukocytes. Eight hours later the white blood cell count was 7500 with 84 per cent polymorphonuclear leukocytes, 14 per cent lymphocytes and 2 per cent monocytes. The hemoglobin was 117 per cent. The urine had a heavy trace of protein, and the specific gravity was 1.014. The Kahn test was doubtful and the Kolmer test negative. Eight hours after admittance to the hospital the serum amylase was 351 mg. At this time the venous pressure was 8 cm. of water, and the electrocardiogram indicated definite myocardial damage.

The patient stated that five years previously he had a similar severe attack of abdominal pain and was very ill for several days. No other illness was admitted.

The patient was given cedilanid intravenously and, approximately twelve hours following hospitalization, the abdomen was explored under a general anesthetic. Several hours following this he became extremely dyspneic and cyanotic. A few hours later the blood pressure was 90/40, and the pulse was rapid and weak. The patient did not respond to supportive therapy and died approximately twenty-one hours following operation and forty hours following admission.

DISCUSSION

Dr. Robert Moore. In view of the pain and rigidity one would need to consider an abdominal crisis. Rigidity ordinarily signifies critical abdominal disease with acute peritoneal irritation. Upper abdominal resistance from thoracic disease usually can be distinguished from actual rigidity. True enough, the rales at the bases, the pretibial edema, the cardiac enlargement and the murmur suggest car-

* Received for publication, February 7, 1948.

diac disease, and the electrocardiogram bears this out. However, the pulse, the blood pressure and the venous pressure are not abnormal. I am inclined to consider the central feature of the crisis to be abdominal rather than cardiac, although I presume the rales at the bases are of cardiac origin. They are bilateral, and there is no mention of dyspnea as a presenting symptom. Thus, an acute pulmonary disease, such as pulmonary embolism, seems unlikely. The red blood cell count and the hemoglobin level suggest more hemoconcentration than the duration of vomiting would lead one to expect. The absence of marked leukocytosis is of little weight in view of the short duration of the attack.

The symptoms do not resemble either obstruction or colic. The pain is not rhythmic and the abdomen is rigid. The more likely classification of the condition is either that of inflammation or perforation. With signs of an abnormal cardiovascular system, an abdominal vascular accident must be kept in mind, although hemoperitoneum by itself should not give true rigidity.

Radiation to the left suggests involvement of an organ in the left upper quadrant. An elevated blood amylase points to the pancreas. A similar episode five years previously might mean recurring gastric ulcer. From the scanty data available, I am inclined to list the probabilities in the following order:

1. Acute pancreatitis, favored particularly by the radiation to the left lumbar region and by the somewhat elevated serum amylase.
2. Perforation of a benign or malignant ulcer, most likely gastric, favored particularly by the rigidity.
3. A vascular accident, such as thrombosis or apoplectic rupture of an upper abdominal vessel, favored particularly by the evidence of a diseased heart. The degree of collapse does not seem sufficient for rupture of an aneurysm.
4. The left kidney, the spleen and the left lobe of the liver are other possible sites of disease.

I would judge that exploratory laparotomy was indicated. The quick demise following operation favors the diagnosis of severe hemorrhagic pancreatitis or a vascular accident rather than a perforation, unless the rapid exitus was due wholly to progression of the cardiac complication.

Dr. Martin Schneider. There are roentgenologic changes suggesting aortic arteriosclerosis with dilatation and tortuosity of moderate extent. The cardiac border seems enlarged to the left. We must take into account, however, the elevated diaphragm, the obesity and probably the failure of the patient to take a complete inspiration. This occurs frequently in a patient with acute abdominal pain. There is an old pleural inflammatory change at the right base, a suggestion of minimal interstitial fibrosis and a few segments of disc-like atelectasis. There is no evidence of free gas in the peritoneal cavity. There are advanced geriatric hypertrophic changes of the lumbar spine. There is no suggestion of increased intra-abdominal fluid. The properitoneal fat line is not disturbed in the left lumbar region.

The findings at the right pulmonary base are not conclusive evidence of abdominal disease. Disc-like atelectasis at the lung base may occur in cases of severe and fatal abdominal trauma, as reported some years ago by Snow. The

reason for such an appearance, however, has never been apparent. I feel reasonably certain that there is no perforation into the major peritoneal cavity. Perforations into the lesser omental sac or retroperitoneal region produce, on occasions, characteristic shadows which also are not seen in this case.

Students' diagnoses and questions. The students considered this case as one of acute pancreatitis, and 15 per cent of them included congestive heart failure in their final diagnoses. The following questions were asked: 1. Please discuss serum amylase determinations; 2. Was Leowi's adrenal test done? 3. Could the episode of five years ago have been acute pancreatitis? 4. Was the abdomen silent? 5. What caused the heart failure?

Dr. Robert Moore. It is most surprising that all of the students settled upon pancreatitis inasmuch as I would not be sure of it. The serum amylase, though elevated, was not really as high as we sometimes see in affections of the pancreas. Consequently, one is inclined to suspect that the diagnosis had leaked out. In a general way, I think it is good to advise that in cases of this sort the surgeon does well to consider more than one diagnosis and to have a second and a third choice, as well as a first. The surgery of the "acute abdomen" is full of surprises, and most of us have learned to our sorrow that it is wise to hedge in the matter and to protect ourselves with alternate diagnoses. In fact, this is the reason for the terms "acute abdomen" and "exploratory laparotomy".

"Could the episode five years previously have been acute pancreatitis?" Yes, certainly it could have been a milder attack of the same nature.

"Was the abdomen silent?" The synopsis does not state, but I would certainly expect the abdomen to be silent. The rigid abdomen is a silent abdomen since inflammation in the peritoneum almost invariably causes an ileus. I saw, a few nights ago, a patient reputed to have peritonitis because "her abdomen was so tender". This patient was rolling and writhing with pain with some paroxysms so severe that she beat the pillow with her fists and vigorously massaged her right flank and groin. Obviously she had right-sided ureteral colic. I do not believe you will ever see a patient with peritonitis threshing about this way. Instead, she will lie perfectly still because the abdomen is too sore for movement. Again, although the patient with progressive hemorrhage may be very restless and may jump about, hemorrhage is not ordinarily accompanied by pain.

"What was the character of the vomitus?" The synopsis does not state, so I presume it was not particularly noteworthy. In this respect I would warn that one should not pay too much attention to the presence of bile in the vomitus. It is present at times in the stomach of many normal individuals, and its appearance in vomitus is not particularly significant.

Dr. Raymond Gregory. It seems that nothing short of acute hemorrhagic pancreatitis or an acute abdominal vascular catastrophe would explain this man's sudden death. Certainly a person would not die from an acute perforated viscus in such a short time following a correction of the perforation. I am interested in this abstract as it relates to the possibility of the findings of pancreatitis. I don't think that these necessarily mean that the process started in the pancreas, although I consider that that is more likely. An ulcer that perforates posteriorly

into the pancreas may give signs that are indistinguishable from processes originating within the pancreas.

A good many implications are made regarding the heart. From the evidence available, I would raise the question whether this man had an elevated pressure originally and then suffered peripheral vascular failure due to shock from his acute abdominal state. The fact that his diastolic pressure was quite depressed is somewhat against this. I am unable to say from the record whether there is any clinical evidence that this man had an enlargement of the heart. There is no documentary evidence here which would enable me to determine the etiologic factor, unless he had a preceding hypertension with a fall in the level of blood pressure as a result of shock. A systolic aortic murmur was present. Nothing is said regarding its intensity, transmission, or whether it was associated with a thrill. The nature of the pulse pressure is distinctly out of line with a severe aortic stenosis. He had a nearly normal pulse pressure, while in serious aortic stenosis there is always a decrease in the pulse pressure, the systolic pressure being at levels of 90 to 100 with normal diastolic levels. I can see no good evidence to lead me to believe that this man had heart failure. The extreme dyspnea and cyanosis that came on subsequent to the operation were probably related to his extreme pain and perhaps to some developing shock-like state.

Cardiac dilatation as a cause of the cardiac enlargement is a possibility, although the heart usually stands up better than this to a short period of stress. So many people at the age of 59 show "slight evidence of myocardial damage" on an electrocardiogram that it really is no just basis for explaining clinical cardiac symptoms. So far as the evidence in the record goes, I don't think I could read much into it from a purely cardiac standpoint.

PATHOLOGIC FINDINGS

Dr. R. H. Rigdon. The body was that of a stocky, middle aged male whose skin was deeply cyanotic. A recent transverse surgical incision, 18 cm. in length with rubber drains, was present in the right upper quadrant. Approximately 1500 cc. of bloody exudate was present in the abdominal cavity. The pancreas was swollen and hemorrhagic, and there appeared to be inflammatory reaction within the stroma and about areas of fat necrosis. The pancreatic ducts were normal in size. The wall of the duct of Wirsung was bile-stained, and its orifice was 2 to 3 mm. in diameter. The gallbladder contained approximately 5 cc. of pale bile and 4 cholesterol-pigment stones, the largest being 0.8 cm. in diameter. There was evidence of chronic inflammation in the wall of the gallbladder. The common bile duct was 0.8 cm. in diameter, but none of the biliary ducts were dilated. In the lower third of the common bile duct there was a tumor mass, 1.5 x 0.5 cm., which was white and friable and did not extend through the duct wall. The ampulla of Vater was located 1.5 cm. from the lower end of the tumor mass. Along the margin of the mucosa that formed the outer portion of the ampulla, there was a papillary tumor mass, 1 x 0.5 cm. Apparently, there was some neoplastic tissue extending along the wall of the common bile duct between the tumor

in the lower end of the common bile duct and the tumor in the ampulla. No neoplastic tissue was found outside of the common bile duct and the ampulla.

The liver weighed 2100 Gm. and gave the appearance of cloudy swelling. The spleen and kidneys were swollen and showed parenchymatous degeneration. The lungs were surrounded by dense, fibrous adhesions and revealed some pulmonary edema and a few petechiae. The heart was of normal size and weighed 345 Gm. The aortic valve leaflets were calcified, producing considerable stenosis. There was fusion of the leaflets to the aorta along their line of attachment. There was only a small amount of atherosclerosis in the aorta associated with a syphilitic aortitis. It should be emphasized that the left ventricle did not show any pathologic changes even in the presence of the aortic stenosis. Except for some petechiae, there were no other lesions found in either the endocardium or myocardium.

Final anatomic diagnosis. Carcinoma of lower third of common bile duct; extension of carcinoma to ampulla of Vater; chronic cholecystitis; cholelithiasis; regurgitation of bile into pancreatic duct; acute pancreatitis with acute localized peritonitis; sanguinopurulent peritonitis (1500 cc.); acute parenchymatous degeneration of viscera; petechiae in gastric mucosa; syphilitic aortitis, chronic; aortic calcific stenosis; fibrous pleural adhesions, bilateral; diverticulum (traction type) in middle third of esophagus.

DISCUSSION

Dr. Rigdon. The tumor apparently arose in the lower part of the common bile duct and extended along the mucosa to reach the ampulla. Such extension has been described by Rolleston. It is difficult to determine the exact site of origin of a tumor that develops in this area; and when the tumor is large, it is impossible to establish the point of origin. It should be remembered that tumors in the region of the ampulla of Vater may arise from the bile duct, pancreatic duct, the mucosa of the ampulla, the head of the pancreas, the mucosa of the duodenum or Brunner's glands. Regardless of the site of origin all produce the same effect, that is, obstruction to the outflow of bile.

Carcinoma of the extra-hepatic bile ducts is considered to be relatively infrequent as compared with carcinoma of other organs. In 2500 autopsies at Cook County Hospital, there were 6 cases of carcinoma of the gallbladder, 4 of the head of the pancreas and 12 of the extra-hepatic bile ducts. In this series of cases, carcinoma of the common bile duct was twice as common as carcinoma of the gallbladder, and 3 times as common as carcinoma of the head of the pancreas. The most frequent location of these extra-hepatic tumors is in the common bile duct. Four were in the lower third and 3 in the mid-portion of the duct.

It is not surprising that no metastases were present in our case. These tumors make their presence known very early by obstruction. Many cases are reported in the literature, however, that show metastases.

The mechanism of acute pancreatitis is of considerable interest and will be discussed by Dr. Tocker. Since the duct of Wirsung is not obstructed, why should pancreatitis develop? In view of the fact that the wall of this duct is bile stained,

I think bile must have extended into it. The mass of tumor, located in the margin of the ampulla, must have caused obstruction to the outflow of bile. Although the exact mechanism is not known in every case, it has been proven that acute pancreatitis will occur if bile is injected into the pancreatic duct. It is of interest with regard to this case to know that Dieulafoy had 11 cases of carcinoma of the ampulla of Vater, and none were accompanied by hemorrhagic pancreatitis.

The aortic stenosis is interesting from the standpoint of etiology. There is usually considerable discussion as to whether such calcified lesions are due to a healed rheumatic process or to arteriosclerosis. Although some pathologists attempt to designate the etiology of these old aortic lesions, I find it unsatisfactory. The presence of syphilitic aortitis in this case presents an interesting problem. I have never seen a case in which syphilis resulted in aortic stenosis. One might ask the question, is it not possible?

Dr. A. M. Tocker. In discussing the pathogenesis of acute pancreatitis, we must consider factors that produce damage both within and outside the pancreas. Just what activates the pancreatic ferments is not definitely known. Within the pancreas, trypsin digests tissue proteins which results in necrosis, erosion of blood vessels (hemorrhage) and split protein products. Suppuration may follow. Lipase digests tissue fat, producing glycerol and fatty acids. The glycerol is absorbed, and the fatty acids combine with calcium to produce calcium soaps which give the areas of fat necrosis their characteristic white appearance. The amylase is absorbed, and its presence in the blood stream is the basis for the serum amylase test. Outside the pancreas the absorbed trypsin and split protein products may induce shock and lead to death. Lipase acts to produce fat necrosis not only in the abdomen, but also in the thorax.

The etiologic factors producing acute pancreatitis may be divided into 3 major groups: (1) Those of non-infectious origin, (2) Those of infectious origin, and (3) Those cases in which a combination of factors must be considered. It is into this latter group that most cases fall since a number of etiologic factors, rather than a single one, seems to be active in the production of the great majority of cases of acute pancreatitis. The case under consideration illustrates this combination of factors. The papillomatous tumor at the ampulla produced a mechanical obstruction resulting in the formation of a common channel permitting the reflux of bile into the duct of Wirsung. It is known that infected bile is a much more potent activator of trypsin than is sterile bile. This patient had chronic cholecystitis and cholelithiasis under which circumstances it is most likely that the bile was infected.

Dr. Paul Brindley. Why don't we have hypertrophy of the left ventricle with such a stenotic aortic valve?

Dr. Rigdon. Anatomically, the valve is stenotic. However, from a functional standpoint there is no evidence that an increased load was placed upon the heart. Why? I am unable to say.

Student. What was the probable nature of the attack the patient had five years ago?

Dr. Rigdon. In view of the chronic cholecystitis and cholelithiasis, I would think it is likely this attack was associated with something in the biliary system.

Student. Do you think that this tumor in the bile duct was present five years ago?

Dr. Rigdon. In my opinion, it was not.

Student. If the bile entered the pancreatic duct because of obstruction, why don't we have a dilatation of the ducts?

Dr. Rigdon. I certainly would not think there has been any obstruction to the outflow of bile preceding this attack of acute pancreatitis. It looks as if the first time that obstruction occurred in the ampulla, as a result of the tumor at the margin of the ampulla, the bile entered the pancreatic duct to start the process of acute pancreatitis. Shock occurred as the result of this diffuse inflammation and is the immediate cause of death. Pulmonary edema and petechiae are consistent with shock.

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EDITORIAL

THE PHOTOGRAPHIC MUSEUM IN THE SERVICE OF PATHOLOGY

Pathologists are by tradition and necessity teachers of medicine, and the pathologic museum is among the important devices which have aided pathologists in carrying out their function as teachers. When the pathologist extended the scope of his activities from the medical school to the so-called "teaching hospital", he built museums in these latter institutions; and when he became a desirable and finally an indispensable part of any modern hospital, the number of museums increased rapidly until the presence or absence of a museum became a factor in determining whether or not, a hospital was suitable for resident training in pathology. Most of us have prepared museum specimens and many of us still look with pride on the long rows of glass jars which represent hours of toil, and often recall interesting cases or personal memories. In scattered museums there are still preserved the specimens from which the original descriptions of pathologic entities were made. We may look upon the very lymph nodes which prompted Hodgkin to describe an important disease, and the thrill is perhaps not diminished when we learn that a century later it was discovered that most of them were not Hodgkin's disease. About the pathologic museum there clings an aura of tradition and associations; to suggest that it no longer is the best kind of museum and that the time has come to replace it, will offend many who have grown up with the old museum. Yet this is precisely what should be done today. To my mind there is no longer any need for a pathologic museum. Its successor is here, the *photographic museum*.

As long as only black-and-white photography was available it offered no serious competition to the old museum. The advent of color photography has changed all this. There is no longer any reason for putting up with the inadequacies and limitations of museum specimens. The new kind of museum illustrates pathology much better than the old, is more versatile, more durable, occupies less space and is less expensive. It serves the expanding functions of the pathologist in the modern hospital. And most important of all, it is not merely a museum of pathology, but a museum of all medicine.

The advantages of the color photograph over the museum specimen are many. Superior retention of the original colors is the most important of these. The fading of the photograph is a small fraction of that induced by fixation. The photograph does not require the repeated attention a museum specimen must have if it is to retain even a semblance of color. Several photographs may show the specimen from varying angles or at different stages of dissection. It is possible to preserve in the photograph the appearances of whole subjects or the relations *in situ*, something which is only rarely possible in a museum specimen and even then only in newborn infants.

The superiority of the photograph in the pathologic conference is immediately apparent. We all remember the poor fellow in the back row who was looking at the specimens from the first case while trying to pay attention to what the

pathologist was saying about the third case. Frequently, too, he was not sure of what he was supposed to see. Contrast this with the picture projected on the screen while the pathologist points out to everyone at the same time the features he wishes to portray. Furthermore, the pathologist can in this way show gross and microscopic features one after the other.

The use of photography in the conference brings up the importance of the changing concept of the place of the pathologist in medicine. No longer is it considered sufficient to show anatomic structure and cytologic detail. The aim of the conference and the function of the pathologist is clinicopathologic correlation. Whether in the conference or in the museum, clinical photographs, pathologic photographs and photomicrographs may now be shown side by side. The pathologist's and clinician's expanding interest in laboratory medicine may be aided by including photographs of exudates and transudates, cultures, results of color or precipitation tests and many others. To these add reproductions of x-rays, electrocardiograms and similar clinical aids, and it is easy to see that for the first time, almost complete clinicopathologic correlation can be not only talked about, but actually shown.

The space occupied by a file of thousands of photographs is minute as compared with the old museum. In our own laboratory, 10,000 lantern slides ($3\frac{1}{4}" \times 4"$) are kept in a corner of an office room, while the discarding of only a few hundred specimens enabled us to more than double the size of the quarters for histologic preparation. Good indexing and cross indexing allow quick access to the slides for a variety of purposes.

An important factor in any museum is cost. Because photography in color is new, its cost, particularly for photographs in larger than miniature size, is likely to be in the forefront of attention. However, because the old museum is accepted, its very high cost may be forgotten. The materials alone which go into the preparation of a specimen about the size of a heart cost as much as the materials for nine color photographs ($3\frac{1}{4}" \times 4"$), while the time required in preparing the museum specimen is very much greater than that required for taking the pictures. The photographic museum is therefore less expensive than the old museum. At this point, it is easy to fall into error. Usually, it does not actually cost less to maintain a photographic museum than a pathologic museum. The reason is that, because of the ease of photography, its better results and its very much greater usefulness, many more pictures are taken than specimens mounted. This should not be allowed to obscure the fact that *for the same number of specimens*, the photographic museum is considerably less expensive.

The introduction of an active photographic department soon dispels the idea that photography is a toy, and in a short time it becomes an every day tool in the active practice of pathology and clinical medicine. When this happens, the necessity for projecting every picture onto a screen whenever one wants to consult the photographic file becomes burdensome. Lantern slides ($3\frac{1}{4}" \times 4"$) in color, although more expensive than miniature sizes, are well worth the extra cost when measured by the greater usefulness for individual study or small

conferences. Furthermore, it is my belief that consistently better pictures may be taken on the larger size, so that there is less waste of film.

I have discussed elsewhere⁵ the aims and criteria of good medical photographs. The administrative problems involved in the setting up of a photographic department have also recently been presented by Sutton.⁶ Only a few points need to be emphasized here. Equipment need not be elaborate at the beginning, and some of it can be improvised easily.¹⁻⁴ The only indispensable piece of equipment is a well trained medical photographer. Good medical photographs can no more be made by technicians who have not been trained for this purpose than good laboratory tests can be turned out by inadequately trained persons. But even the best trained photographer needs medical direction. It is my belief that the best results are not obtained when the photographer is in complete charge of his department, any more than when a technician is in charge of the clinical laboratory. Since the laboratory is the focus of correlation and since the pathologist is usually the spark plug for the educational activities of the hospital, I believe that in most institutions it would be advantageous to place the pathologist in charge of the photographic department.

The pathologist may, of course, learn to take his own pictures, but I am not sure that it is wise that he expend his time in the purely technical aspects of photography. His main concern should be to see that the picture portrays the medical features properly. As in the clinical laboratory, his time should largely be spent on the meaning rather than in the technical performance.

The success of a medical photograph depends on how well the pathologic changes are shown. The photograph is a tool in the study and teaching of pathology and medicine. This, and not merely the collection of pretty pictures, justifies the building of a photographic museum. So useful is this tool that in time no hospital will be without its photography department. And, in the growth of this enlarged conception of the museum, the pathologist will, as usual, be the moving spirit.

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SELECTED ABSTRACTS

Discussion on Amoebiasis. Tr. Roy. Soc. Trop. Med. and Hyg., 41: 55-91, 1947.

In opening the discussion, Dr. C. M. Wenyon, the presiding officer, spoke of the increased interest in the subject of amoebiasis stimulated by wars. There is, of course, a greater number of cases at home than in normal times because of the returning soldiers who have been infected in foreign lands where the disease is endemic. Dr. Wenyon would like to know what becomes of the chronic cases and of the carrier who is refractive to treatment. He recalled the "supposed epidemic of amoebic dysentery" in Gallipoli in the first world war which proved to be one of bacillary dysentery and raised the question, "Do epidemic outbreaks of amoebic dysentery occur?" He believes the Chicago epidemic was mainly a bacillary one in a group of people in which the *E. histolytica* carrier rate was high. He noted that the question of carriers is one of peculiar difficulty, since there is such a large number of cyst-passers and there are relatively few cases of amoebic dysentery. Apparently asymptomatic carriers will develop the disease on going to the tropics. Is this due to the strain of amoebae they were carrying or to a new strain? If due to the original strain, what were the changes in the intestine to cause the amoebae to become pathogenic? He didn't know whether *E. histolytica* can live in the intestine of man without giving rise to lesions.

On the question of how many examinations are necessary to exclude amoebiasis, his own method is to ask for an ordinary specimen, one not procured following a saline purge, once weekly for six weeks. He doubted that cultural methods would disclose many infections which could not be detected by a careful microscopic examination. He also doubted that complement-fixation tests will help very much. He believes that red blood cells occur so seldom in association with *E. coli* that for all practical purposes this possibility can be ignored. He has never seen it.

Concerning the advisability of treating the symptomless carrier, he believed that having discovered an infection, the physician has the responsibility of trying to get rid of it but should not institute wholesale examinations with a view to the detection and treatment of every carrier. Cases of acute amoebic dysentery, of course, demand immediate attention.

Dr. F. Murgatroyd, the next speaker, stated that direct microscopy of freshly passed stools remains the basic diagnostic procedure. There seems to be a dispute as to the value of the saline purge. Culture methods may help but sometimes cultures are negative when the stool is known to contain parasites. He has not been able to confirm the results of those who have recommended "provocative emetine", and believes that the idea of a dose of emetine increasing the number of parasites is theoretically unsound. He has not had the aid from proctoscopy that others have claimed for the procedure. He cited Craig's work on complement-fixation as a diagnostic aid but says that the test does not appear to be one that is either readily applicable or reliable. He believes that we lack precise knowledge regarding the best amoebicidal therapy and how it should be employed. British practice favors a combination of drugs, e.g., emetine bismuth iodide, 2 or 3 grains by mouth, with retention enemata of 2½ per cent chiniofon daily for ten to twelve days followed by carbarsone 0.25 grams by mouth twice daily for ten days. Sometimes, this course is preceded by parenteral injection of 1 grain of emetine hydrochloride. As for the treatment of asymptomatic carriers, he is in accord with Wenyon in that they should be treated where practical.

Sir Phillip Manson-Bahr, the next speaker, considered emetine or emetine bismuth iodide the mainstay of treatment of amoebiasis, whether chronic or acute. He disagreed with the previous speaker in regard to the usefulness of the proctoscope. He thinks it a most useful instrument as a diagnostic aid and that it often succeeds where other methods fail. He emphasized his stand on this subject by stating, "Direct inspection of the mucosa, direct examination of the biopsy material from the mucosa, constitutes the best method of diagnosis". He thinks little of radiography as a means of diagnosis except, perhaps, in

caecal amocbiasis where little assistance can usually be obtained by endoscopy or examination of the feces.

The next speaker, Dr. G. T. Stewart, spoke on "added bacterial infection" in amocbiasis and showed that on examination of the stools from patients with amoebic dysentery, the paracolon bacilli and *Streptococcus fecalis* were more prevalent than in the stools of controls. He refers to the work of Hargreaves who reported encouraging results in treating relapsing cases of amoebiasis with penicillin and sulpha drugs.

Rochester, New York

W. S. THOMAS

A Modification of the Zinc Sulphate Centrifugal Flotation Technique for the Concentration of Helminth Ova and Protozoan Cysts in Feces. J. M. WATSON. Ann. Trop. Med. and Parasitol., 41: 43, 1947.

Take a specimen of feces the size of a pea, break it up in a centrifuge tube, add distilled water, centrifuge for three minutes at 1500 r.p.m., decant the supernatant. Repeat this procedure twice, then fill the tube with $\text{ZnSO}_4 \cdot 7 \text{H}_2\text{O}$, 33 per cent aqueous solution (sp. gr. 1.18) to a point where the meniscus just reaches the rim. The tube is then covered with a circular cover slip which has been smeared with Meyer's glycerin-albumin mixture, taking care not to trap an air bubble. Centrifuge again for three minutes at 1500 r.p.m. and remove the cover slip carefully and quickly and place face down on a slide on which has been placed a drop of iodine solution. Great superiority is claimed for this method over that where a pipet or loop has been used.

W. S. THOMAS

Zinc Sulphate Flotation of Feces. RONALD ELSDON-DEW. Tr. Roy. Soc. Trop. Med. and Hyg., 41: 213, 1947.

While doubt as to the efficacy of this method has been expressed by some, Elsdon-Dew is not one of these. He believes that this method is becoming more popular and in his hands it has been most successful as is evident from the following figures: the overall gain over the direct examination was 179 per cent. For *E. histolytica* it was 175.5 per cent, for *G. lamblia* 500 per cent, for *A. duodenale* 538 per cent and for *I. bütschlii* 577 per cent. Moreover, oxyuris ova were found by the method, whereas they had not been seen on direct examination. He finds the method suitable for specimens containing much mucus and, of course, it is worthless for trophozoites. He suggests the use of a hydrometer to determine specific gravity rather than to rely on weighing, since he finds variation in different batches of zinc sulphate.

W. S. THOMAS

The Plasma Cellular Reaction and Its Relation to the Formation of Antibodies in Vitro. AS-TRID FAGRAEUS. J. Immunol., 58: 1-14, 1948.

In a previous paper the author described the cellular changes in the spleen after intravenous injection of horse serum into previously sensitized rabbits. Two to three days after the reinjection, cells of characteristic appearance were found in the reaction centers, the periphery of the lymph follicles and in the red pulp. These cells, which were believed to originate from the reticulum cells, were called transitional cells; for, some days later, in the same locations appeared numerous, somewhat smaller cells with the characteristics of immature plasma cells. Simultaneously, the antibody titer of the blood showed considerable increase. At the peak of the serum titer curve there was an increased number of mature plasma cells.

Similar experiments using intravenous *S. typhi* gave results quite similar to those just stated.

Very minute portions of red pulp and follicular tissue were removed from the spleen and placed in normal rabbit serum and Tyrode's solution and incubated. The culture fluid was then diluted and mixed with *S. typhi* O and H antigen solution and incubated. The agglutinin titer was definitely and considerably higher with the red pulp culture.

In other experiments, pieces of red pulp were cultured with transfer to fresh mediums every twelve hours. The antibody titer increased during the first twelve hours and reached its peak in the second twelve hours. Thereafter it decreased.

S. typhi organisms injected intravenously were found after five to twelve minutes to be 5 to 8 times as numerous in the red pulp as in the follicles. The number fell more rapidly in succeeding minutes in the red pulp, indicating more rapid destruction. Histologic examination showed few bacteria in the follicles but large numbers of them in the periphery of the follicles and in the red pulp where the plasma cells are found in such numbers after the injection.

It is believed that antibodies may be formed by the reticulo-endothelial cells which pass through developmental changes, ending in the mature plasma cell.

Dallas, Texas

J. H. BLACK

Accidental Electrocution: With Direct Shock to the Brain Itself. W. E. C. DICKSON. J. Path. and Bact., 59: 359, 1947.

Accidental electrocution of a 45 year old woman is reported by Dickson with unusual brain changes. During an operation on the trigeminal nerve, there was a short-circuit, and 240 volts were directly applied to the temporal area of the brain. There was immediate unconsciousness, which lasted until death twenty-one and one-fourth hours later.

The naked-eye and microscopic appearances of the brain were striking, with cortical radial striations like those of "the gills of a mushroom". These were believed produced by the bubbling action of steam and gas following the intense heat and electrolytic action of the current. Except for a report by Hassin (Arch. Neurol. and Psychiat., 30: 1046, 1933), there is no similar finding recorded.

Fort Wayne, Indiana

S. M. RABSON

Spinous Process Puncture. A Simple Clinical Approach for Obtaining Bone Marrow. J. PHILIP LOGE. Blood, 3: 198-206, 1948.

The author carefully describes in detail a method for obtaining marrow by aspiration of the spinous process of either the third or fourth lumbar vertebra. The total nucleated cells and differential studies are compared with those obtained simultaneously from specimens from the sternal marrow. The technic appears simple and has the advantage that the patient does not see the preparation and actual puncture performed. Of particular interest to this reviewer are the similar values for cellular constituents when obtained from the sternum, iliac crest or vertebral spinous process. The procedure is recommended when a "dry" sternal tap is obtained or when the patient is apprehensive.

Brooklyn

LEO M. MEYER

The Heterologous Growth of Cancer of the Human Prostate. M. S. HOVENANIAN AND C. L. DEMING. Surg., Gynec. and Obst., 86: 29-35, 1948.

Human prostatic adenocarcinoma was transplanted into the anterior chamber of the guinea pig eye. The original morphology of the human tumor was reproduced in 6 serial transfers, for which a total of 84 guinea pigs was used. An interesting sex difference became apparent, inasmuch as 47 per cent of successful "takes" of the transplants occurred in 68 male guinea pigs, whereas not a single growth persisted in 15 female animals. Likewise, castrated males failed to develop "takes". In one instance, administration of massive doses of testosterone to a female guinea pig resulted in the successful growth of the transplant. No significant changes in the activity of acid phosphatase were found in the blood of animals with growing transplants. By means of the histologic staining reaction, the tumor transplants themselves were shown to have lost the ability of producing acid phosphatase.

This study is another example of utilization of heterologous tumor transfer for investigations into the biology of neoplasms (cf. an earlier paper of the same authors, abstracted

in this Journal 17: 842, 1947). In this specific instance, the dependence of human prostatic cancer on hormonal factors was corroborated; on the other hand, autonomous tumor growth persisted in spite of loss of an enzymic factor (acid phosphatase).

Chicago

KURT STERN

The Nature of Acid-Fastness. DIRAN YEGIAN AND ROBERT J. VANDERLINDE. J. Bact., 54: 777-783, 1947.

Many theories have been proposed to explain the acid-fast property of the tubercle bacillus, but no satisfactory explanation has yet been given. It is suggested that the property is dependent upon the permeability of the cytoplasmic membrane. During the staining procedure, by the Ziehl-Neelsen technic, fuchsin enters the cell through the cytoplasmic membrane and is not removed by the acid-alcohol used in the method. A small portion of the dye acts as though firmly bound by the cytoplasm and gives the organism only a very faint pink color. The rest of the dye can be accumulated in beads and acts as free dye; it is to this form that the usual color of the stained acid-fast bacillus is attributed.

The authors show that mycobacteria will exhibit acid-fastness and beading even after extraction of the free lipids. These extracted lipids are only faintly acid-fast. Certain acid-fast structures, other than tubercle bacilli, (some mushroom spores) have been shown to contain only small quantities of lipids.

Chicago

BEN FISHER

Variation in the Prothrombin Test Technique. BEN FISHER. Am. J. M. Sc., 215: 39-41, 1948.

Variations in the prothrombin test technic were studied in relation to the order of mixing the plasma and the reagents. A statistical study is presented on data derived from studies made on "normal" patients, using the Quick one-stage technic and the Link-Shapiro method. The actual standard deviation is shown to be less by Quick's method.

BOOK REVIEWS

American Medical Research, Past and Present. By RICHARD H. SHRYOCK, Ph.D., Professor of History and Lecturer in Medical History, University of Pennsylvania; Acting Director, American Council of Learned Societies. 350 pp. \$2.50. New York: The Commonwealth Fund, 1947.

Dr. Shryock has reviewed the history of medical research in America from the middle of the eighteenth century. The development of medical science in this country is discussed in relation to the formative influences of British, French and German medicine. One of the valuable features of the book is that medical research is discussed in relation to the development of the social sciences and industrial research. The sources of research support are discussed with regard to the changing economic and social background. It should be of interest to all pathologists that an unbiased professional historian as Dr. Shryock attributes such great credit for the development of medicine and research to pathology and also a matter of pride that William H. Welch is noted at the top of all medical scientists in this country when considered from this broad viewpoint.

Various trends as well as different fields of research are discussed, and particular attention is given to research in the medical schools. Considerable detailed information is given concerning the support of medical research by foundations and government agencies. A section is also devoted to the future of research; this should be of interest to all scientists in view of changing social and economic conditions. This method of organization of scientists and scientific investigations for World War II is described, as well as a brief résumé of what was accomplished. The author has done a complete job and nothing of importance seems to have been omitted.

The book has been well edited and documented; however, on page 111 Dr. William Snow Miller is referred to as Dr. William Snow.

The accurate and concise information contained in this volume should be of interest to anyone concerned with or actively participating in medical research.

Madison, Wisconsin

D. MURRAY ANGEVINE

Textbook of Pathology. Ed. 5. By WILLIAM BOYD, M.D., Professor of Pathology and Bacteriology of the University of Toronto. 1049 pp., 500 illus., 30 colored plates. \$10.00. Philadelphia: Lea and Febiger, 1947.

In the fifth edition the author continues to make a serious effort to correlate disturbed physiology and morbid anatomy, thus accomplishing to a real degree his goal of presenting pathology as an introduction to medicine. The highly readable style of the author makes the text attractive to the student, and at the same time the subject is presented in an authentic and inclusive manner. The integration of excellent, instructive, colored plates with the text is an improvement over the last edition. There is relative freedom from inconsistency and ambiguity. One exception noted is that the author accepts the European concept of the non-thrombotic occlusion of capillaries in the stasis of frost-bite, a viewpoint which is inconsistent with his definition of a thrombus.

The text has been brought up to date in many chapters by the addition of new sections dealing with a wide variety of subjects, especially in the field of respiratory pathology, the anemias, the diseases of the endocrine system and of the liver. However, the author does not depart from his goal of supplying a basic text of pathology intended primarily for the undergraduate student.

The section on allergy has been reinserted in the text. Some sections of the book have been rewritten. Among the important topics receiving expanded or modified discussion are: carcinogenesis in its relation to enzymes and viruses, silicosis, necrosis and cirrhosis of the liver, and the Rh factor in relation to congenital hemolytic diseases.

While this volume is intended primarily for the student at the undergraduate level, it is recommended as a basic text and minor reference work for the physician who maintains continuing interest in pathology.

Detroit

A. L. AMOLSCH

The Pathology of Nutritional Disease. Physiological and Morphological Changes Which Result From Deficiencies of the Essential Elements, Amino Acids, Vitamins, and Fatty Acids. By RICHARD H. FOLLIS, JR., M.D.; Formerly Associate Professor of Pathology, Duke University School of Medicine, Durham, North Carolina; now Associate Professor of Pathology, The Johns Hopkins Medical School, Baltimore, Maryland. 310 pp., 8 tables, 71 figs. \$6.75. Springfield, Illinois: Charles C Thomas, 1947.

In this book the author has set out "to gather together the available information which deals with the physiological and morphological changes occurring naturally or produced experimentally which accompany deficiencies of one or more of the 40 odd nutrients now known to be essential". The book is divided into six parts, with sections on dietary deficiencies in general, the essential elements, essential amino acids, vitamins and essential fatty acids. In each chapter, after a short historical introduction, the biologic and biochemical relationships are discussed and then the pathologic effects described. A short outline of the effect of the particular deficiency in man is added. In the last part of the work, the pathologic anatomy of specific tissues is discussed. This particularly interesting section summarizes and critically reviews the anatomic changes in the various organs, as they may occur in deficiencies due to the various essential nutrients. A selected bibliography covering 791 papers concludes the work.

The author, who himself has made many valuable contributions to the subject, has admirably accomplished his aim. Of the pathologic effects described as occurring in nutritional deficiencies in general, only those are accepted which are based on clear-cut evidence. It is, therefore, not surprising that most of the data are derived from animal experimentation and comparatively few established facts from deficiencies in man. A special appeal of the book to the reviewer lies in the emphasis which the author gives to the many defects in our present knowledge. The disproportionate lack of knowledge concerning anatomic changes occurring in nutritional deficiencies, compared to the relatively large amount of physiologic and biochemical information, is made very evident. This fact represents a real challenge to the clinical and experimental pathologist. It can surely be expected that in future editions of the book much new material will be added.

Although the book explicitly deals only with the effects of nutritional deficiencies, occasional reference might have been made to the effect of a surplus of certain nutrients. For instance, not only deficiency in l-cystine but also overdoses of l-cystine may lead to hemorrhagic necrosis of the liver which cannot be prevented by choline.

The printing and illustrations are excellent.

Brooklyn

M. WACHSTEIN

Biochemistry For Medical Students. Ed. 4. By WILLIAM VEALE THORPE, M.A., Ph.D., Reader in Chemical Physiology, University of Birmingham (England). 496 pp., 36 illus. \$5.00. Baltimore: The Williams & Wilkins Company, 1947.

The fact that this excellent little book is now in its fourth edition scarcely ten years after the publication of the first edition attests both to its popularity as a textbook of biochemistry and to the author's zeal in keeping the material up-to-date. The book has not suffered in conciseness or clarity as a result of its several revisions and remains, as the author originally intended it, "as concise as is consistent with accuracy".

The book is written primarily as a synopsis of biochemistry for workers in the medical sciences or a supplementary textbook for medical students. Prominence is, therefore, given to discussions of the blood, urine and feces. Special attention is also devoted to the principles of nutrition and to the composition of foodstuffs—a feature of practical importance. The new chapter in this edition on the use of isotopes in biochemical investigations should also be of value to the busy clinical worker.

In a few places, the book has not quite kept pace with recent advances in the field, no doubt because of the rapid progress of the past year or two. For example, there is no more than a scant mention of folic acid and nothing on the importance of glutamine in ammonia transport. The author also, apparently, still subscribes to the now questionable view that "failure to utilize carbohydrate means incomplete combustion of fat" (p. 232). Also, the statement that the excretion of hippuric acid is a test of the detoxicating power of the *kidney* (p. 459) is certainly open to question.

Despite these minor criticisms, the reviewer highly recommends this book as a concise, readable synopsis of biochemistry especially useful for the busy clinical or laboratory worker.

Detroit

JAMES M. ORTEN

Poisons. Their Isolation and Identification. By FRANK BAMFORD, B.Sc., Late Director of the Medico-Legal Laboratory, Cairo; Second edition revised by C. P. STEWART, M.Sc., Ph.D., Reader in Clinical Biochemistry, University of Edinburgh. Senior Biochemist, Royal Infirmary, Edinburgh. 304 pp., 23 illus. \$5.00. Philadelphia: The Blakiston Company, 1947.

The second edition of this book, prepared by C. P. Stewart, is largely unchanged from the first edition which was written by F. Bamford, B.Sc., late director of the medico-legal laboratory, Cairo, Egypt and published in 1940. The new material consists principally of the addition of 2 paragraphs describing the spectograph and spectroscop, three and one-half pages on gaseous poisons, additional qualitative tests for lead, copper, thallium, nickel, chlorate, bromine and cannabis indica, the iodometric determination for arsenic, the precipitin test for toxalbumins and a more detailed discussion of digitalis glycosides. Emphasis throughout is placed on the forensic rather than the industrial aspect of toxicologic chemistry. Although the sections on metallic poisons and barbiturates are only reasonably well covered, those dealing with alkaloids, glucosides and the detection of unusual and obscure poisons are to be recommended. Chapter X, entitled a "Systematic Scheme for the Identification of Alkaloids", is commendable.

The prefatory remarks in the first edition indicate the purpose for which the book is written . . . "it is intended as a laboratory manual for all chemists who have to deal with cases of poisoning". Despite the non-uniformity in the treatment of subjects and the lack of an adequate bibliography, the book adequately accomplishes this purpose, and for this reason it is recommended for laboratories in which toxicologic analyses are done. Purchase of the second edition is hardly necessary for those in possession of the first edition.

Boston

WALTER W. JETTER

Studies On the Influenza-A Epidemic of January-March 1941 at Gronigen (Holland). By J. A. R. VAN BRUGGEN, M.D., L. BIJLMER, M.D., W. A. HOEK, M.D., J. MULDER, M.D., AND L. J. ZIELSTRA. 79 pp., 44 figs., 27 tables. \$2.00. Leiden: H. E. Stenfert Kroese's Uitgevers-Maatschappij N. V., 1947.

This article represents a reprinting of a thesis published earlier in Holland. Beginning with a brief review of the epidemic distribution of influenza in the years 1936-46, the major part of the report deals with the clinical aspect of influenza as it was encountered. Particular attention is given to a number of bacterial complications which are of interest especially since a fatal case in this instance was associated with *Staphylococcus aureus*, an observation which is becoming of greater significance in fatalities from influenza. The authors suggest the possibility of microscopic examination of fixed sections of sputum for interpretation of the type of injury occurring.

The section on the identification of virus adds little new although it is an excellent effort to correlate the etiology with other data. The work constitutes an interesting addition to the current literature, particularly as to the occurrence of influenza in another country.

Ann Arbor, Michigan

THOMAS FRANCIS, JR.

Pathologisch-anatomische Untersuchungen über Leberzirrhose bei Säuglingen und Kleinkindern (infantile Leberzirrhose) mit endemischer Häufung. By DR. HERMANN GÖGL, Innsbruck. 155 pp., 29 figs. \$7.75. New York: Grune and Stratton, Inc., 1947.

The author studied 24 cases of infantile liver cirrhosis in the Pathologic Institute of the Innsbruck University Medical School. The age of the infants ranged from 2 months to 15 months and most of the cases occurred in the village of Kitzbühel. The clinical histories and the anatomic and histologic findings are given in detail, the latter illustrated by 28 photomicrographs, the reproduction of which is unsatisfactory due to the poor quality of the paper.

The disease is characterized anatomically by an extensive epithelial degeneration of the liver cells with formation of basophilic and eosinophilic globules. The fibrous tissue is excessive, and the lobular arrangement is completely lost. A varying degree of proliferation of bile ducts and of inflammatory cell reaction is mentioned. The sublobular veins may be completely obliterated following a peculiar inflammation of their wall.

No specific bacterial cause could be found. The occasional presence of inclusion bodies suggests to the author the probability of a virus infection. No definite association with epidemic hepatitis could be established.

The fact that the disease was almost never observed in first-born children, that in one family the two first-born children remained well, while the following four all died from liver cirrhosis, and the observation that in families with two wives all children of one mother died of liver cirrhosis, while those of the other mother remained well, make one suspicious that infantile liver cirrhosis may be the result of neonatal hemolytic disease. It is to be regretted that the author did not investigate his interesting material from this angle. There is no mention of Rh studies on parents or infants by the author.

Wichita, Kansas

C. A. HELLWIG

Sexual Behavior in the Human Male. By ALFRED C. KINSEY, Professor of Zoology; WARDELL B. POMEROY, Research Associate; AND CLYDE E. MARTIN, Research Associate, Indiana University. 804 pp., 162 tables, 173 figs. \$6.50. Philadelphia: W. B. Saunders Company, 1948.

This book, the first of its kind, is a remarkable scientific achievement that can easily mark an epoch in the evolution of medical and social attitudes toward human sexual behavior.

It is based upon searching, controlled and confidential interviews with 12,000 males, carefully selected to comprise representative cross sections for the various economic, occupational, social, religious, age and geographic distributions. The approach is scientific and statistical, but more than half the book is devoted to simple, readable, interesting, factual analysis and explanations of the findings. There is no drawing of moral interpretations, merely a presentation of facts.

The findings recorded by the authors will force a significant alteration in currently held views and misunderstandings of this subject which is much tabooed, even among medical men. General dissemination of the knowledge in this book will unquestionably render society a significant service by relieving people of many of those intense conflicts basic to personality disturbances and psychosomatic ills.

The book should be required reading for a college education and a source book for teaching from infancy to adulthood. It is an absolute requisite for all students of biology, psychology, sociology and medicine.

The authors plan to continue this study until they have recorded the facts for a total population of 100,000 males. Additionally, a comparable study is in progress for females, and a companion volume on the human female will be forthcoming shortly.

One can safely say that this book will have a permanent beneficial influence upon human welfare.

Eloise, Michigan

MILTON H. ERICKSON

The Epithelia of Woman's Reproductive Organs. A Correlative Study of Cyclic Changes. By GEORGE N. PAPANICOLAOU, M.D., Ph.D., Professor of Clinical Anatomy, Cornell University Medical College; HERBERT F. TRAUT, M.D., Professor of Obstetrics and Gynecology, University of California Medical School; AND ANDREW A. MARCHETTI, M.D., Associate Professor of Obstetrics and Gynecology, Cornell University Medical College. 52 pp., 22 plates, 20 in color, one large colored chart. \$10.00. New York: The Commonwealth Fund, 1948.

This volume represents "a comprehensive report on the cytology of the epithelial structures of the normal human female genital tract. Special emphasis is placed on the interdependence of the epithelial elements and the correlation of the cytologic changes in the epithelium of each portion of the tract at various phases of the menstrual cycle. A new interpretation of functional changes is presented, and a baseline of the normal is drawn against which gynecologists can more easily recognize and identify aberrations.

"This monograph is based on a ten year study conducted jointly at the Departments of Anatomy, and Obstetrics and Gynecology of the Cornell University Medical College with material obtained at the Woman's Clinic of the New York Hospital. Jointly undertaken by a group of workers with biologic, physiologic and gynecologic interests, this study represents a cooperative effort to contribute to an understanding of the functional characteristics of the female organs of generation by describing, interpreting and correlating the cytologic aspects. The individual cell structure and tissue organization of each section of the genital tract is described with special attention to the changes which occur in each during the phases of the menstrual cycle.

"The correlation of cytologic changes is graphically illustrated in a large colored chart showing the interrelationships among various sections of the tract during each phase of the menstrual cycle. In addition, twenty-one pages of colored illustrations, both photomicrographs and drawings are included for complete and precise description of the epithelia of woman's genital tract."

This text is concise and clearly written, the format is attractive, and the photomicrographs are of superb quality. Its chief value for pathologists lies in the ready reference which may be made to the photomicrographs of the various normal tissues at different stages in the menstrual cycle.

Disputed Paternity Proceedings. Ed. 2. By SIDNEY B. SCHATKIN, Assistant Corporation Counsel of the City of New York. 614 pp. \$10.00. Albany, N. Y.: Matthew Bender & Company, 1947.

The first edition was reviewed in the *Journal* (15: 33, 1945). The present edition was brought up to date and was enlarged by 172 pages. Most of them (117) have been added to Part I, which is the portion of main interest to the clinical pathologist. There are four new chapters in this part. Chapter V on the Rh-Ir blood types presents the application of the discoveries of Landsteiner and Wiener to paternity proceedings. Chapter VII deals with blood tests, an effective rebuttal of the presumption of legitimacy. The unerring accuracy of blood tests is shown convincingly in Chapter VIII. In the last new chapter of Part I, the illegitimate child's birth certificate is discussed. Also new is Appendix II on Domestic Relations Law, Article VIII.

It is gratifying to find that the statement in the first edition that only a hematologist is competent to carry out forensic blood grouping tests has been changed in favor of a serologist, in line with a suggestion of this reviewer.

Pathologists who want full and competent information on this important subject will find it clearly presented in this valuable monograph. The concluding statement of the review of the first edition can be well used again: "It is hoped that the book will be instrumental in calling attention in suitable places to the existence of the only available scientific and reliable means of eliminating injustice to men innocently accused." Other states

would do well to follow the example of New York, Wisconsin, Ohio, New Jersey, South Dakota, Maryland and North Carolina.

Chicago

I. DAVIDSOHN

Skin Manifestations of Internal Disorders (Dermadromes). By KURT WIENER, M.D., Dermatologist, Mount Sinai Hospital, Deaconess Hospital, Saint Michael's Hospital, Milwaukee, Wisconsin. 690 pp., 386 illus., 6 color plates, 6 tables. \$12.50. St. Louis: The C. V. Mosby Company, 1947.

Dermatologic Clues to Internal Disease. By HOWARD T. BEHRMAN, M.D., Assistant Clinical Professor of Dermatology, New York University College of Medicine; Adjunct Dermatologist, Mount Sinai Hospital and Beth Israel Hospital; Associate Dermatologist, Hillside Hospital; Diplomate of the American Board of Dermatology and Syphilology; Fellow of the American Academy of Dermatology and Syphilology. 165 pp., 118 illus. \$5.00. New York: Grune & Stratton, 1947.

The subject of these two books has always intrigued the physician. With the advent of and the ever increasing emphasis on laboratory diagnostic methods, cutaneous manifestations of internal diseases have been shoved into the background along with so many other phenomena of disease which our medical forebears had used successfully in their diagnostic armamentarium.

By way of justifying his effort, Wiener states in the preface: "A recent systematic presentation of the skin manifestations of internal disorders does not exist and the author hopes to fill this gap with the present book." He begins by introducing a new term "dermadromes" to mean "true skin manifestations of internal disorders". Wiener defines "dermadrome" somewhat suggestively as "literally, fellow traveler on the skin".

Eight of the book's 43 chapters are devoted to cutaneous manifestations of systemic infections. Other chapters deal with helminthic diseases, tuberculosis, lesions of controversial tuberculous etiology, leprosy, intoxications, ageing, internal cancer, metabolic disorders (four chapters), disorders of circulation, kidneys, endocrine glands (eleven chapters), blood and blood forming organs (three chapters), nervous system (five chapters), gastrointestinal tract, liver, pancreas and respiratory tract.

The treatment of the subject is lucid, critical, thorough and exhaustive. There are 386 well selected and executed illustrations and six plates with 6 color illustrations on each, the latter of uneven quality. The references, numbering 3110, are listed at the foot of the pages. There are seven instructive tables summarizing acute exanthems, diffuse pigmentations, diffuse yellow pigmentations, the skin in relation to age, hemorrhagic diseases and pruritus. The index is well organized.

Behrman's little volume aims to meet the same need as the more ambitious and bulky monograph just described. The material is treated in brief paragraphs arranged alphabetically. There is no attempt to give complete information. Behrman's book will be of service as a convenient source of essential information. Its merits cannot be compared with the scholarly treatise of Wiener. It has 118 well-done illustrations, but no references. The price of \$5.00 seems somewhat high considering its size.

This reviewer is not qualified to pass on the purely dermatologic aspects of either book. He approached them with the idea of seeing what they might offer to a pathologist. In the course of reading, he has become aware of the sad fact that as a pathologist he has sorely neglected, at the autopsy table, the skin as a source of important pathologic enlightenment. While it is true that pathologists are less in need of dermatologic clues to internal diseases, some of us have excluded ourselves from an interesting, stimulating and particularly significant field that may offer opportunity for fruitful research. Wiener's book, especially, may open to us new vistas at the autopsy table. It is from this point of view that the books are recommended to readers of this Journal.

I. DAVIDSOHN

NEWS AND NOTICES

NEW CANCER JOURNAL

A new journal, *CANCER*, sponsored by the American Cancer Society, will shortly make its initial appearance. Every phase of the cancer problem will be covered, with major emphasis on clinical aspects. Dr. Fred W. Stewart, of Memorial Hospital, New York, will be the Editor-in-Chief, assisted by an editorial advisory board of 50 authorities.

TEXAS SOCIETY OF PATHOLOGISTS

On January 25, 1948, the Texas Society of Pathologists had its annual meeting at Galveston, Texas. A business meeting was held at the Galvez Hotel, followed by a banquet. Officers elected for the year, 1948, were: President, Dr. W. W. Coulter, Sr., Houston; Vice-President, Dr. Charles Phillips, Temple; President-Elect, Dr. John F. Pilcher, Corpus Christi; Secretary, Dr. C. T. Ashworth, Dallas. During the afternoon a seminar was held and conducted by Dr. Paul Brindley in which microscopic sections of pathological lesions were commented upon and studied. Dr. T. J. Curphey, President of the American Society of Clinical Pathologists, was guest of the society. The next annual meeting will be held in Dallas, January 30, 1949.

AMERICAN ASSOCIATION OF BLOOD BANKS

The next annual meeting of the American Association of Blood Banks will be held in Buffalo, New York, August 26-28, 1948, immediately following the meeting of the International Hematology Society. Membership in the Association is of two classes, an institutional membership which is available to ethical, independently operating and policy-making, non-profit institutions, hospitals registered by the American Medical Association engaged in blood banking, and an individual membership which is open to any person interested in blood banking. The Secretary of the Association is Miss Marjorie Saunders, LL.B., 3301 Junius Street, Dallas 1, Texas.

REPRINTS OF "THE HOSPITAL LABORATORY" AVAILABLE

Reprints of "The Hospital Laboratory", prepared as a part of a series of guide materials for hospital planning, may be obtained from Dr. J. R. McGibony, U. S. Public Health Service, Division of Hospital Facilities, Washington 25, D. C.

NEW YORK STATE MEDICAL SOCIETY

A motion was brought before the House of Delegates of the New York State Medical Society by Dr. Stephen Curtis of Troy, the delegate from Troy and also a member of this Society, at the request of the New York State Society of Pathologists and reads as follows.

"Whereas, it has been established by the American Medical Association that the practice of Pathology is the practice of Medicine; and

"Whereas, many of the recent advances in the field of medicine are distinctly attributable to the contribution of the workers in the field of laboratory research and the practical application thereof in the field of clinical medicine; and

"Whereas, the practitioner of medicine welcomes and utilizes fully the scientific help supplied by these practitioners of laboratory medicine; and

"Whereas, in the hospitals of this country there now exists an intolerable situation by which the natural expansion of Laboratory Medicine is being retarded:

(a) Through the improper and arbitrary control of the scientific and administrative efforts of this group of medical practitioners; and

(b) By the extensive economic exploitation by the institutions of the scientific efforts of this group; and

"Whereas, it is the feeling that the present existing situation will diminish the number of younger men entering the ranks of this speciality, thus seriously depleting a growing and increasingly useful branch of medical practice; now be it

"Resolved, that the House of Delegates of the Medical Society of the State of New York give cognizance to the existing situation; and be it further

"Resolved, that the situation be carefully studied by the Society with the avowed purpose of developing a new pattern of practice of the specialty of laboratory medicine in the hospitals, placing such practice on the same broad basis as now governs the relationship of medical specialties with the institutions of this state, and that a committee be appointed by the President with the approval of the Council to carry out this study."

The Council, having received this matter from the House of Delegates, referred it both to the Joint Committee of the Hospital Association of New York and the Medical Society of the State of New York, and to the Committee on Economics of the Medical Society of the State of New York. This matter was considered under the guidance of the Committee on Economics on March 3, 1948. It was agreed that preparation of a bill for the 1949 New York State Legislature will be undertaken. The object will be to define the relationships of medical specialists with hospitals and other institutions.

OHIO SOCIETY OF PATHOLOGISTS

The winter meeting of the Ohio Society of Pathologists was held February 28, 1948, in the Department of Pathology, Cincinnati General Hospital. The program consisted of a general discussion of "Surgical Pathology of the Alimentary Tract" by Dr. Nathan C. Foot. New additions to the Slide Library, consisting of lesions of the gastro-intestinal tract, were also discussed by Dr. Foot.

JAMES EWING SOCIETY

A two day program of recent advances in the field of cancer was presented on January 16 and 17, 1948, in the Memorial Hospital, New York. Separate sessions were devoted to clinical investigation in cancer, indications for radical surgery and pathological studies.

TECHNICAL SECTION

GROWTH OF PATHOGENIC FUNGI ON A NEW CULTURE MEDIUM*

M. L. LITTMAN, PH.D.

*From the Department of Pathology and Bacteriology, Tulane University
School of Medicine, New Orleans, Louisiana*

A new agar culture medium for the primary isolation of pathogenic fungi from all types of clinical specimens was recently described.⁴ In this medium crystal violet and streptomycin were used as selective bacteriostatic agents while oxgall was used to restrict spreading of fungus colonies.

The completed medium contained in distilled water, 1 per cent dextrose, 1 per cent granular peptone, 1.5 per cent dehydrated oxgall, 2 per cent agar, and 0.001 per cent crystal violet† with no pH adjustment of the medium. Sterilization was accomplished at 10 to 12 lb. pressure at 115–117.7 C. for fifteen minutes, and the agar was cooled to approximately 46 C. before adding sufficient streptomycin sulfate to provide a final concentration of 30 units of the antibiotic per cc. of medium.

The inhibitory action of the poured medium to gram-positive and gram-negative bacteria was found to be unaltered by several weeks' storage at 28 C. and 37 C. The growth of 22 species of pathogenic fungi and 11 species of saprophytic fungi in our stock culture collection, as well as that of numerous aerial saprophytic types, was found to be unaffected by the streptomycin in the concentration employed.^{5, 6}

In order for laboratory workers to utilize oxgall streptomycin crystal violet agar (OSCV) to better advantage for the culture of fungi from clinical specimens, more information was needed concerning the rate of growth of fungi on the medium. For this purpose, then, experiments were conducted with large and small inoculums of fungi on OSCV agar and Sabouraud's dextrose agar. Twenty-three species of pathogenic fungi and 11 of saprophytic fungi, listed in Tables 1, 2 and 3 were employed in this study.

EXPERIMENTAL

Growth from Large Inoculums

Three to five mm. fragments of fourteen-day-old cultures of fungi maintained on Sabouraud's agar were transplanted to slants of OSCV and Sabouraud's agar (4 per cent dextrose, 1 per cent peptone, 2.5 per cent agar, pH 5.6) which were incubated at 30 C. Cultures were examined every twenty-four hours, and the elapsed time was recorded for the first macroscopic signs of growth. In two

* Received for publication, January 3, 1948.

† Crystal violet (indicator): dye content, 91 per cent; National Aniline Division, 40 Rector Street, New York City. Stock solution: 1.25 grams dissolved in 25 cc. of 95 per cent ethyl alcohol and kept in a tightly stoppered bottle. For use: 0.2 cc. added/liter of medium.

TABLE 1

INCUBATION IN DAYS AT 30 C. FOR FIRST MACROSCOPIC SIGNS OF GROWTH OF FUNGI ON
OXGALL STREPTOMYCIN CRYSTAL VIOLET AGAR AND SABOURAUD'S DEXTROSE
AGAR WHEN 3-5 MM. FRAGMENTS OF MYCELIUM ARE INOCULATED

ORGANISMS*	SABOURAUD'S DEXTROSE AGAR**		OSCV AGAR†	
	Trial 1	Trial 2	Trial 1	Trial 2
Pathogenic species				
<i>Candida albicans</i>	1	1	1	1
<i>Cryptococcus neoformans</i>	1	1	1	1
<i>Geotrichum</i> sp.	1	1	1	1
<i>Monosporium apiospermum</i>	2	3	2	2
<i>Sporotrichum schenkii</i>	2	3	2	2
<i>Coccidioides immitis</i>	2	3	2	3
<i>Trichophyton rubrum</i>	2	3	2	3
<i>Trichophyton mentagrophytes</i>	3	3	3	3
<i>Trichophyton violaceum</i>	5	5	3	5
<i>Trichophyton schoenleinii</i>	3	4	3	5
<i>Trichophyton sulfureum</i>	3	3	3	3
<i>Microsporum gypseum</i>	2	3	2	2
<i>Microsporum canis</i>	3	3	3	3
<i>Microsporum audouinii</i>	3	3	3	3
<i>Epidermophyton floccosum</i>	3	3	3	3
<i>Hormodendrum compactum</i>	3	4	3	5
<i>Hormodendrum pedrosoi</i>	3	3	3	5
<i>Phialophora verrucosa</i>	5	3	3	5
<i>Blastomyces dermatitidis</i> (A-387)‡	3	4	4	5
<i>Blastomyces dermatitidis</i>	3	4	3	5
<i>Blastomyces brasiliensis</i>	6	6	6	8
<i>Histoplasma capsulatum</i>	3	3	4	6
<i>Nocardia asteroides</i>	5	5	No growth	No growth
Nonpathogenic species				
<i>Candida candida</i>	1	1	1	1
<i>Rhizopus nigricans</i> (+) and (-)	1	1	1	1
<i>Penicillium expansum</i>	1	1	1	1
<i>Penicillium notatum</i>	1	1	1	1
<i>Scopulariopsis brevicaulis</i>	1	1	1	1
<i>Neurospora sitophila</i>	1	1	1	1
<i>Fusarium</i>	2	1	1	2
<i>Alternaria</i>	2	3	1	2
<i>Cladosporium</i>	2	3	2	2
<i>Mucor mucedo</i>	2	1	2	2
<i>Aspergillus herbariorum</i>	2	3	2	3

* Furnished by Dr. Norman F. Conant, Duke University, Durham, N. C. and Dr. Georges Knaysi, Cornell University, Ithaca, N. Y.

** Four per cent dextrose, 1 per cent peptone, 2.5 per cent agar, pH 5.6.

† Oxgall streptomycin crystal violet agar.

‡ Isolated from a fatal case of North American blastomycosis.

TABLE 2

GROWTH OF PATHOGENIC AND SAPROPHYTIC FUNGI ON OXGALL STREPTOMYCIN CRYSTAL VIOLET AGAR AND SABOURAUD'S DEXTROSE AGAR FROM SERIAL DECIMAL DILUTIONS OF ORGANISMS

ORGANISMS	INCUBATION IN DAYS AT 30 C. FOR APPEARANCE OF COLONIES								COLONY COUNT							
	Sabouraud's Dextrose Agar*				OSCV Agar				Sabouraud's Dextrose Agar				OSCV Agar			
	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵
Pathogenic species																
<i>Candida albicans</i>	1	1	1	1	1	2	2	2	>300	>300	>300	112	>300	>300	>300	118
<i>Cryptococcus neoformans</i>	2	2	2	2	2	2	2	2	>300	>300	140	22	>300	>300	132	14
<i>Geotrichum sp.</i>	1	1	1	1	1	1	1	2	>300	222	37	3	>300	290	36	2
<i>Monosporium apiospermum</i>	1	2	2	2	1	2	2	3	Cf†	>300	175	30	Cf	>300	174	27
<i>Sporotrichum schenkii</i>	2	2	2	3	2	3	3	4	Cf	>300	>300	61	Cf	>300	>300	110
<i>Coccidioides immitis</i>	3	3	3	3	3	3	3	3	Cf	Cf	170	19	Cf	>300	186	20
<i>Trichophyton rubrum</i>	2	3	3	4	2	3	3	3	Cf	>300	200	21	>300	>300	190	21
<i>Trichophyton mentagrophytes</i>	2	2	3	3	2	3	3	3	Cf	Cf	>300	56	>300	>300	>300	34
<i>Trichophyton violaceum</i>	3	5	7	7	3	3	4	5	>300	>300	78	12	>300	>300	115	12
<i>Trichophyton schoenleinii</i>	6	7	7	—	7	7	7	—	>300	97	13	2	>300	43	4	1
<i>Trichophyton sulfurcum</i>	2	2	3	2	2	2	2	3	Cf	Cf	49	40	>300	>300	47	16
<i>Microsporum gypseum</i>	2	2	2	2	2	2	2	2	Cf	>300	79	12	>300	>300	82	15
<i>Microsporum canis</i>	3	3	4	4	3	3	4	8	Cf	48	7	1	>300	73	11	1
<i>Microsporum audouinii</i>	3	4	5	—	3	4	7	—	68	15	2	0	121	8	1	0
<i>Epidermophyton floccosum</i>	2	3	4	—	4	5	—	—	72	15	3	0	55	6	0	0
<i>Hormodendrum compactum</i>	7	8	12	15	4	5	6	6	>300	>300	109	12	>300	>300	100	12
<i>Hormodendrum pedrosoi</i>	6	6	8	10	5	6	6	7	>300	>300	60	4	>300	>300	36	3
<i>Phialophora verrucosa</i>	3	3	5	5	3	4	5	6	>300	>300	101	12	>300	>300	90	10
<i>Blastomyces dermatitidis</i>	3	4	5	5	5	6	7	8	>300	>300	43	4	>300	>300	34	3
<i>Blastomyces brasiliensis</i>	6†	—	—	—	6†	—	—	—	4	0	0	0	4	0	0	0
<i>Histoplasma capsulatum</i>	2	2	6	—	—	—	—	—	95	6	1	0	0	0	0	0
<i>Nocardia asteroides</i>	3	3	3	3	—	—	—	—	>300	>300	>300	200	0	0	0	0

TABLE 2—Continued

ORGANISMS	INCUBATION IN DAYS AT 30 C. FOR APPEARANCE OF COLONIES								COLONY COUNT							
	Sabouraud's Dextrose Agar*				OSCV Agar				Sabouraud's Dextrose Agar				OSCV Agar			
	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵
Nonpathogenic species																
<i>Candida candida</i>	1	1	1	1	2	2	2	2	>300	>300	>300	300	>300	>300	>300	300
<i>Rhizopus</i> <i>nigricans</i>	1	1	1	1	1	1	1	1	Cf	Cf	10	4	>300	220	28	5
<i>Penicillium</i> <i>expansum</i>	3	3	3	4	3	3	4	7	Cf	>300	100	10	>300	>300	93	5
<i>Penicillium</i> <i>notatum</i>	2	2	2	2	2	2	2	2	Cf	Cf	>300	68	>300	>300	>300	66
<i>Fusarium</i>	1	2	2	2	2	2	2	2	Cf	24	5	5	>300	45	5	4
<i>Alternaria</i>	1	2	3	—	2	4	5	—	Cf	8	1	0	20	3	C	0
<i>Neurospora</i> <i>sitophila</i>	2	2	—	—	2	2	—	—	Cf	Cf	0	0	7	2	0	0
<i>Cladosporium</i>	2	3	4	5	2	4	6	7	Cf	>300	80	11	Cf	>300	>300	38
<i>Aspergillus</i> <i>herbariorum</i>	2	3	—	—	3	5	—	—	68	4	0	0	39	6	0	0
<i>Scopulariopsis</i> <i>brevicaulis</i>	2	2	2	—	2	2	2	—	>300	73	1	0	>300	33	8	0

C, contamination.

* 2 per cent dextrose, 1 per cent neo-peptone, 2 per cent agar, pH 5.6.

† 10⁻¹ dilution.

‡ Cf, confluent growth.

separate trials it was found that, with the exception of *Nocardia asteroides* which failed to develop on OSCV agar, growth was initiated on the two mediums in approximately the same time by each fungus in our collection of 33 species (Table 1). The failure of *N. asteroides* to grow on OSCV agar was expected because of its known sensitivity to streptomycin. On OSCV agar fully grown colonies of fungi were uniformly smaller, discrete and *non-spreading* in comparison to their spreading character on Sabouraud's agar.

It was also noted that species of fungi varied considerably in the lag period of growth which extended from the time of transplant until the first signs of growth. For instance, a lag period of six days was exhibited by *Blastomyces brasiliensis* on Sabouraud's dextrose agar and eight days on OSCV agar before visible growth appeared, while growth of *Geotrichum* was initiated on both mediums in only one day. As there is scant information in the literature regarding this period of delay, the data presented in Table 1 may serve as a useful guide for technicians working with OSCV agar.

Growth from Diluted Inoculums

Generous segments of twenty-five-day old cultures of fungi, previously maintained on Sabouraud's dextrose agar, were thoroughly fragmented in sterile

TABLE 3

SIZE IN MILLIMETERS OF COLONIES OF PATHOGENIC AND SAPROPHYTIC FUNGI ON OXGALL STREPTOMYCIN CRYSTAL VIOLET AGAR AND SABOURAUD'S DEXTROSE AGAR DEVELOPING FROM SERIAL DILUTIONS OF ORGANISMS

ORGANISMS	SABOURAUD'S DEXTROSE AGAR*								OXGALL STREPTOMYCIN CRYSTAL VIOLET AGAR							
	Incubation in Days at 30 C.								Incubation in Days at 30 C.							
	2	4	6	8	10	15	21	56	2	4	6	8	10	15	21	56
Pathogenic species																
<i>Candida albicans</i>	3	4	6	6	6	6	6	6	1	3	4	5	6	6	6	6
<i>Cryptococcus neoformans</i>	1	5	7	9	10	10	10	10	pp	4	6	7	9	10	10	11
<i>Geotrichum sp.</i>	9	24	38	47	Cf	Cf	Cf	Cf	5	10	16	22	27	40	55	57
<i>Monosporium apiospermum</i>	2	10	14	Cf	Cf	Cf	Cf	Cf	pp	4	8	13	19	19	19	24
<i>Sporotrichum schenkii</i>	1	3	5	8	11	15	16	17	pp	pp	1	2	3	4	7	17
<i>Coccidioides immitis</i>	—	9	20	Cf	Cf	Cf	Cf	Cf	—	5	11	16	25	Cf	Cf	Cf
<i>Trichophyton rubrum</i>	pp	2	8	12	13	Cf	Cf	Cf	pp	1	2	5	6	8	12	24
<i>Trichophyton mentagrophytes</i>	1	4	11	15	20	Cf	Cf	Cf	pp	1	3	6	8	11	14	18
<i>Trichophyton violaceum</i>	—	pp	pp	pp	2	6	8	20	—	pp	1	2	2	4	5	11
<i>Trichophyton schoenleinii</i>	—	—	pp	1	3	6	16	20	—	—	—	pp	2	5	7	22
<i>Trichophyton sulfureum</i>	3	6	8	14	19	24	24	24	1	2	7	10	14	17	22	32
<i>Microsporum gypsum</i>	1	6	18	23	30	Cf	Cf	Cf	pp	3	5	9	11	14	20	22
<i>Microsporum canis</i>	—	3	14	27	42	75	85	85	—	pp	6	10	19	29	39	85
<i>Microsporum audouinii</i>	—	2	8	17	22	39	66	80	—	1	3	4	6	15	22	70
<i>Epidermophyton floccosum</i>	1	2	7	13	21	38	Cf	Cf	—	pp	2	4	6	9	9	11
<i>Hormodendrum compactum</i>	—	—	—	pp	pp	pp	1	13	—	pp	pp	1	2	4	5	10
<i>Hormodendrum pedrosoi</i>	—	—	pp	pp	2	6	12	32	—	—	pp	1	2	4	8	21
<i>Phialophora verrucosa</i>	—	pp	1	3	5	8	8	11	—	pp	pp	1	2	3	6	8
<i>Blastomyces dermatitidis</i>	—	pp	1	4	5	8	15	45	—	—	pp	1	3	5	8	30
<i>Blastomyces brasiliensis</i>	—	—	2	5	7	9	10	26	—	—	2	4	5	8	9	13
<i>Histoplasma capsulatum</i>	pp	2	5	10	11	15	21	52	—	—	—	—	—	—	—	—
<i>Nocardia asteroides</i>	—	pp	1	2	2	2	3	3	—	—	—	—	—	—	—	—
Nonpathogenic species																
<i>Candida candida</i>	3	3	3	3	4	5	5	5	pp	1	3	3	3	5	5	5
<i>Rhizopus nigricans</i>	38	Cf	Cf	Cf	Cf	Cf	Cf	Cf	3	4	5	Cf	Cf	Cf	Cf	Cf
<i>Penicillium expansum</i>	—	1	2	4	4	6	10	13	—	pp	1	2	2	3	6	16
<i>Penicillium notatum</i>	3	5	7	11	16	17	17	17	pp	2	4	6	6	8	8	8
<i>Fusarium</i>	12	28	43	54	68	Cf	Cf	Cf	4	11	18	22	32	50	70	90
<i>Alternaria</i>	3	7	20	34	46	56	71	82	3	10	11	18	19	26	26	32
<i>Neurospora sitophila</i>	Cf	Cf	Cf	Cf	Cf	Cf	Cf	Cf	9	Cf	Cf	Cf	Cf	Cf	Cf	Cf
<i>Cladosporium</i>	pp	1	1	2	3	6	7	7	pp	pp	pp	1	1	3	6	8
<i>Aspergillus herbariorum</i>	pp	7	11	13	17	20	32	36	—	1	2	4	6	9	16	16
<i>Scopulariopsis brevicaulis</i>	1	8	10	13	15	22	52	66	1	5	9	13	15	26	26	32

Cf, confluent growth; pp, pin point.

* 2 per cent dextrose, 1 per cent neo-peptone, 2 per cent agar, pH 5.6.

physiologic saline and serial decimal dilutions prepared therefrom. Measured quantities of each dilution were spread on the surface of OSCV and Sabouraud's dextrose agar which were incubated at 30 C. and examined at regular intervals for total number and size of colonies.

The relative productivity and sensitivity of both types of culture mediums in developing fungal colonies from diluted inoculums is shown in Table 2. It is apparent that most species of fungi tested, except *N. asteroides*, developed approximately the same number of colonies on both mediums. With certain exceptions the time required for the first appearance of fungal colonies from a given inoculum was approximately the same on both types of culture mediums. *Hormodendrum compactum*, *Hormodendrum pedrosoi* and *Trichophyton violaceum* produced visible colonies from all serial dilutions sooner on OSCV agar than on Sabouraud's agar, while *Epidermophyton floccosum* and *Blastomyces dermatitidis*

TABLE 4

RELATIVE PRODUCTIVITY OF CULTURE MEDIUMS IN ISOLATION OF FUNGI FROM CLINICAL SPECIMENS

SPECIMENS		SABOURAUD'S DEXTROSE AGAR*			OSCV AGAR		
Type	Number Examined	Fungal Types	Number Fungal Colonies	Specimens with Fungi of Known Pathogenicity	Fungal Types	Number Fungal Colonies	Specimens with Fungi of Known Pathogenicity
Feces.....	20	7	163	0	13†	504	0
Sputum.....	11	7	201	0	11‡	327	0
Skin, hair, nails.....	16	5	19	2	30	259	8
Total.....	47	19	383	2	54	1090	8
Increase over Sabouraud's dextrose agar, per cent.					284	284	400

* 2 per cent dextrose, 1 per cent neo-peptone, 2 per cent agar, pH. 5.6.

† In order of frequency: *Geotrichum*, *Candida*, *Penicillium*, *Aspergillus*, *Rhodotorula*, *Hormodendrum*, *Mucor*, *Paecilomyces*, *Fusarium*, *Trichoderma*, *Mycoderma*, *Monilia nigra*, *Phoma sp.*

‡ In order of frequency: *Candida*, *Fungi imperfecti*, *Aspergillus*, *Hormodendrum*, *Mucor*, *Penicillium*, *Scopulariopsis*, *Alternaria*, *Rhodotorula*.

produced visible colonies sooner on the latter medium. *N. asteroides* and *Histoplasma capsulatum* failed to grow on OSCV agar from highly diluted inoculums, although growth of the latter mold occurred when a fragment of culture, 3 to 5 mm. in diameter, was inoculated (Table 1).

Data on the incubation time required by species of pathogenic fungi to appear on OSCV agar on primary isolation from infected human discharges and exudates is not yet recorded in the literature. If this time should be equal to that required by a highly diluted fungal suspension to develop visible colonies on agar, then the data contained in Table 2 may be useful as a temporary guide to laboratory workers.

During the first three weeks of incubation of plates containing highly diluted inoculums, spreading activity of both pathogenic and saprophytic fungi was greatly restricted on OSCV agar as compared with Sabouraud's agar.

Since the criterion of "visible growth" which formed the basis of data contained

in Tables 1 and 2 varies with many individual factors, it was felt that measurement of colonies of each fungal species at regular time intervals was a more precise indicator of rate of growth. These data are presented in Table 3. During the first month of incubation at 30 C., the size of colonies on OSCV agar, of the majority of species of fungi, measured approximately one-half that on Sabouraud's dextrose agar (Fig. 1). In fifty-six days, however, 21 of the 32 species of fungi had produced colonies on OSCV agar equal in size to those on Sabouraud's agar, as illustrated by *Microsporum canis* (Fig. 2). This was taken to mean that the nutrients available for potential growth of fungi were approximately equal on both types of culture mediums.

CLINICAL USE OF THE NEW FUNGUS CULTURE MEDIUM

Sputum and Feces

Twenty specimens of feces and 11 of sputum were selected at random at the Clinical Diagnostic Laboratory of Charity Hospital, New Orleans. Generous quantities of specimens were vigorously spread with sterile swabs over the surface of OSCV agar and Sabouraud's dextrose agar plates, which were then incubated at 30 C.

At the end of five days' incubation the majority of Sabouraud's agar plates which had been streaked with feces was overgrown with gram-negative bacteria (Fig. 3A), and those inoculated with sputum were covered with luxuriant growth of gram-positive bacteria (Fig. 3C). A few mold and yeast colonies present on Sabouraud's agar were submerged in part by the heavy growth of bacteria. Examination of OSCV agar on the other hand revealed that 28 of the 31 plates had developed no bacterial colonies whatsoever, but instead numerous yeast and mold colonies (Fig. 3B and D). Colonies of fungi were small, discrete and *non-spreading*. Pure cultures were obtained with ease. Three remaining OSCV agar plates, which had been inoculated with fecal suspensions, developed luxuriant bacterial growth due to the presence in the specimens of streptomycin-tolerant bacteria.

Extension of incubation to ten days permitted overgrowth of Sabouraud's plates by saprophytic fungi, but it did not change the appearance of OSCV plates from that at five days' incubation, save for a moderate increase in size of fungal colonies.

Colonies of fungi developing on both types of culture mediums were counted, transplanted in pure culture and subsequently identified. When the data were tabulated (Table 4), it was found that OSCV agar had produced approximately three times as many colonies and species of fungi from fecal suspensions and sputum as Sabouraud's agar. As an example of the type of results obtained, it may be noted that one fecal specimen streaked on Sabouraud's agar developed luxuriant bacterial growth but no fungi, while OSCV agar failed to grow bacteria, and revealed growth of the following fungi with indicated numbers of colonies: *Geotrichum* 1, *Trichoderma* 2, *Candida* 30, *Rhodotorula* 40.

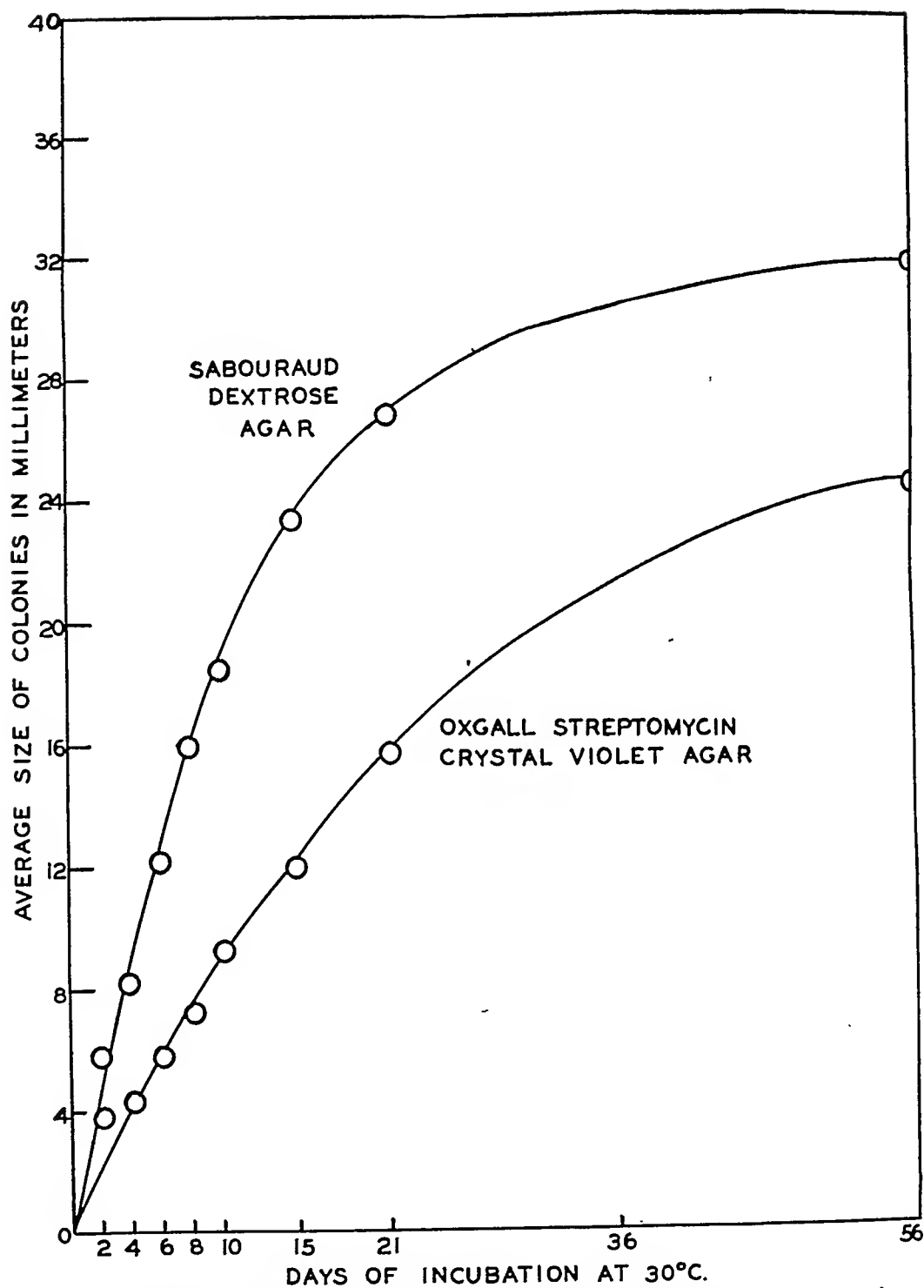


FIG. 1. Average colony size of 30 species of pathogenic and saprophytic fungi on oxgall streptomycin crystal violet agar and Sabouraud's dextrose agar.

Skin and Nail Scrapings and Hair

Sixteen specimens received no treatment with disinfecting agents; instead they were distributed directly over the surface of OSCV agar and Sabouraud's dextrose

agar plates. After eight days' incubation at 30 C. the majority of Sabouraud's plates were overgrown with either bacteria or saprophytic fungi or both, while OSCV agar plates yielded numerous, discrete, small colonies of fungi and no bacterial colonies. From a total of 16 specimens of skin and nail scrapings and hairs, only two dermatophytes were isolated with Sabouraud's agar while eight were obtained with OSCV agar.

DISCUSSION

Although only 47 clinical specimens of feces, sputum, skin scrapings and hair were examined with OSCV agar, the results obtained indicate that this medium holds considerable promise as a diagnostic tool for the primary isolation of fungi from specimens containing a mixed bacterial and fungal flora. Continuing studies which greatly extend these observations (to be published) have corroborated the observed high productivity of OSCV agar in the isolation of pathogenic fungi, especially dermatophytes.

From a scanty literature on the subject of fungal flora of the gastro-intestinal tract, one gains the impression that many fecal specimens examined were reported not to yield fungi,¹ while other studies indicate that only a few different genera of fungi are found in the gastro-intestinal tract.^{2, 7, 8} In the present experiments, when OSCV agar was employed for the examination of 20 fecal specimens, a surprisingly large number of fungi was isolated, *i.e.*, 504 colonies of 13 different fungal genera (Table 4). Most fecal specimens streaked on OSCV agar yielded colonies of fungi in varying numbers. Therefore, in the light of the high productivity of OSCV agar in the isolation of fungi from the feces, the entire study of the fungal flora of the gastro-intestinal tract deserves re-evaluation. Recent studies by Felsenfeld,³ which correlated the incidence of *Geotrichum* in the gastro-intestinal tract of institutionalized patients with "food upsets", help to focus attention on fungi as hitherto unrecognized agents of disease and also to raise the pressing issue as to which species of fungi comprise the normal flora of the gastro-intestinal tract as contrasted with abnormal or pathogenic flora. The etiologic role of fungi in acute and chronic disorders of the upper and lower respiratory tract is also deserving of investigation. With the use of a medium like OSCV agar, such studies should be greatly simplified.

Note. A scum which forms on the surface of the sterile agar medium consists principally of calcium phosphate, the formation of which is accentuated by excessive or prolonged heating. In the prepared dehydrated medium, the materials have been processed to avoid scum formation. The dehydrated medium* contains all the elements of the formula except streptomycin and may be sterilized at 15 lb. pressure (121 C.) for fifteen minutes without formation of a surface precipitate. This medium, to which streptomycin was added, has been tested and found satisfactory for general use.

* Known as Bacto-Littman Oxgall Agar and prepared by Difco Laboratories, Detroit, Michigan.

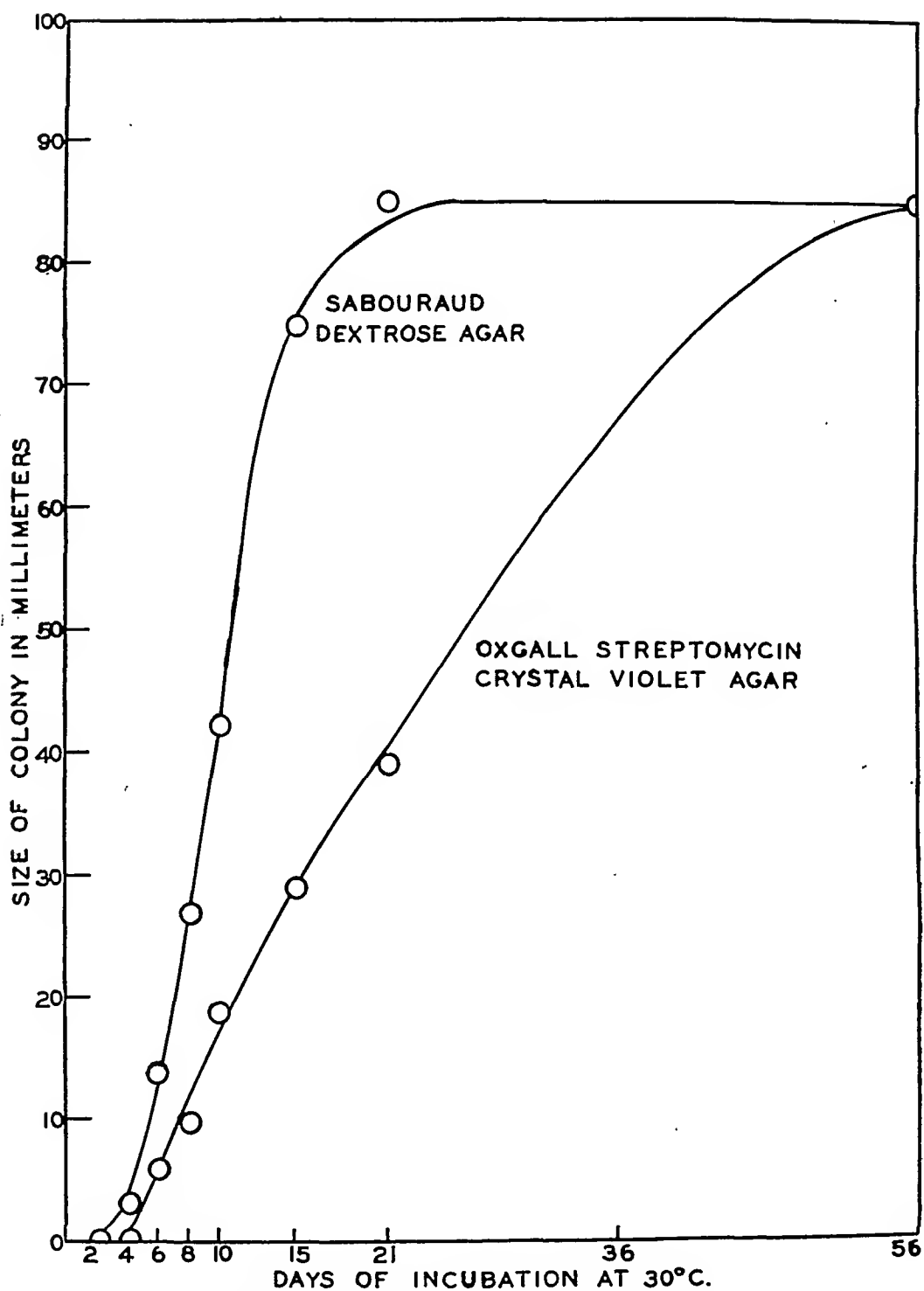


FIG. 2. The size of colonies of *Microsporum canis* on oxgall streptomycin crystal violet agar and Sabouraud's dextrose agar.

SUMMARY

Oxgall streptomycin crystal violet agar* permitted growth of 33 species of fungi from fragments of inoculums, 3 to 5 mm. in diameter, at the same speed as

Sabouraud's dextrose agar. *Nocardia asteroides* failed to grow because of sensitivity to streptomycin. The new medium also produced the same number of colonies of fungi from highly diluted pure culture suspensions as did Sabouraud's

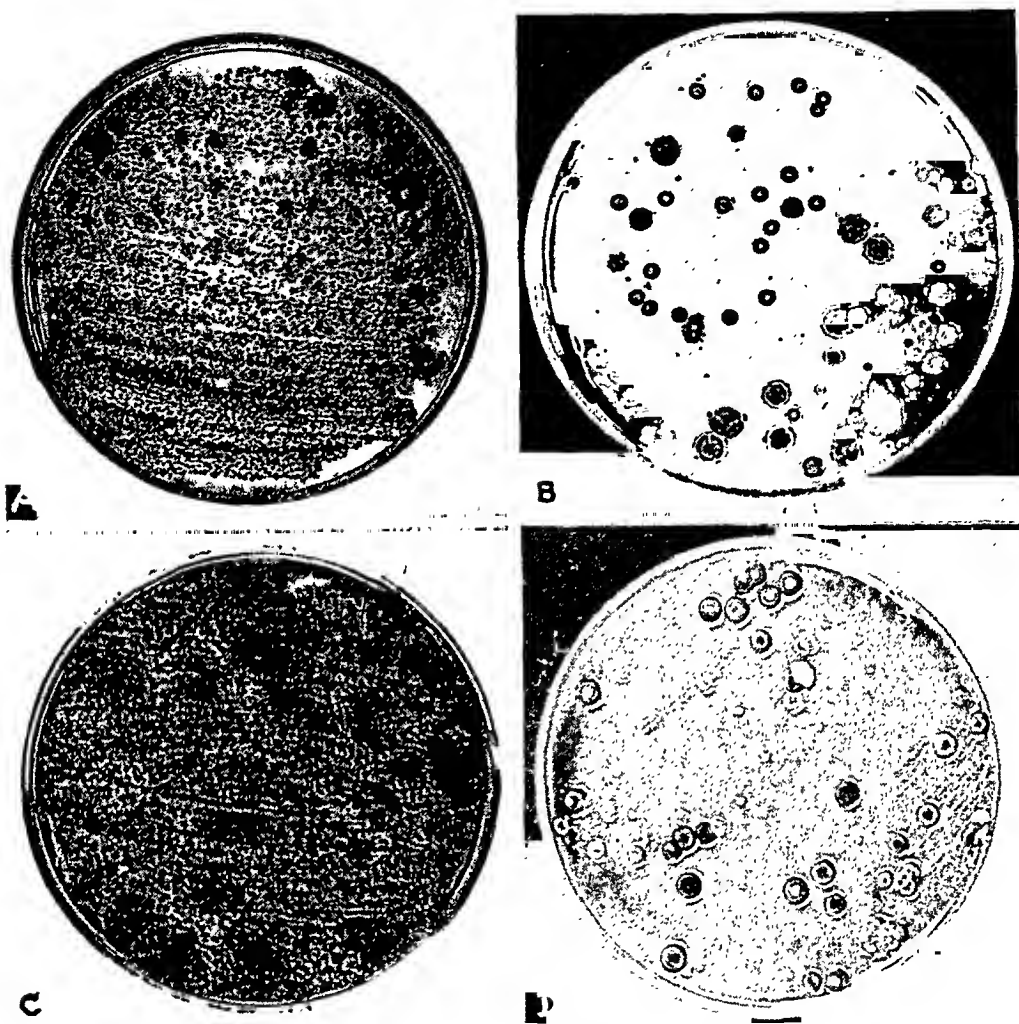


FIG. 3. Showing comparison of growth of fungi on Sabouraud's dextrose agar (A and C) and oxgall streptomycin crystal violet agar (B and D).

A and B. Mediums inoculated heavily with fecal suspension and incubated at 30 C. for five days. A. Sabouraud's dextrose agar. Note abundant bacterial growth which hindered fungal development. B. Oxgall streptomycin crystal violet agar. Note minimal bacterial growth and abundant non-spreading colonies of *Aspergillus*, *Penicillium*, *Monilia nigra* and *Candida*.

C and D. Mediums inoculated heavily with sputum and incubated at 30 C. for five days. C. Sabouraud's dextrose agar. Note numerous colonies of staphylococci which are covered by growth of *Penicillium*. D. Oxgall streptomycin crystal violet agar. Staphylococci are inhibited; numerous mold colonies are small, discrete and non-spreading.

agar. *Histoplasma capsulatum* from suspensions of highly diluted pure culture failed to grow on the oxgall agar, although vigorous growth was obtained with fragments of mold 3 to 5 mm. in diameter.

The majority of fungi tested produced colonies on the new medium measuring approximately one-half the size of colonies on Sabouraud's agar at the end of the first month of incubation and equalled their size by the fifty-sixth day. Spreading of all fungi was considerably curtailed on the new medium.

Use of the new medium for the cultivation of fungi from feces, sputum, skin scrapings and hair showed a three-fold increase in the total number of fungal species and colonies isolated as with Sabouraud's agar. Four times as many pathogenic dermatophytes were isolated with the new medium as with Sabouraud's agar. Five hundred and four colonies of 13 different genera of fungi were isolated from 20 specimens of feces using the new medium.

A record is presented of the size of colonies of pathogenic fungi on the oxgall agar and the normal speed of growth of numerous species.

Acknowledgments. The author wishes to thank Dr. E. S. Moss, Charity Hospital, New Orleans, Louisiana, for laboratory facilities, Dr. T. Laskaris for identification of many species of fungi and Dr. M. F. Shaffer for many helpful suggestions.

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PLASMA CONCENTRATIONS FOLLOWING INTRAMUSCULAR INJECTIONS OF VARIOUS DOSES OF PENICILLIN*

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The therapy of many diseases now routinely includes the use of penicillin.^{4-6,9,10} The dosage and the route and frequency of administration of this antibiotic vary with the infection under treatment, or with personal choice and expediency. In some cases the infecting organism is isolated from the patient and tested for its sensitivity to penicillin *in vitro*. If this has been determined, frequently it is considered desirable to employ a schedule of treatment that will yield plasma penicillin concentrations that are equivalent to, or preferably higher than, the *in vitro* sensitivity of the infecting organism. Since the rapidity with which this drug is excreted makes it impossible to maintain a constant level of the antibiotic agent in the blood stream by means of any form of intermittent therapy, the time during which plasma penicillin concentrations remain above any selected level during such treatment depends chiefly on the size of the dose that is given. Intramuscular injection commonly is employed as a means of administering penicillin, and information concerning the relationship between the amount of penicillin injected and the resulting plasma penicillin concentrations should make it possible for the clinician to use penicillin more intelligently and efficiently.

The application of such information regarding "dose-response" to therapeutic procedures requires not only a knowledge of the approximate values of average plasma penicillin concentrations but also an appreciation of the factors that contribute to the individual variations to be expected following the intramuscular injection of a given dose of penicillin. Moreover, it is essential that there be an understanding of the variations that may occur within the limits of the assay procedure used in order to determine from laboratory reports whether or not the schedule of treatment is producing the desired plasma concentrations of penicillin. Reports in the literature of specific instances of penicillin therapy have included illustrative values on assays determined at various times after aqueous penicillin has been administered intramuscularly,^{3, 7, 8, 11, 13, 14} but, in general, these have not represented a systematic effort to obtain data covering the effects of a wide range of dosages at specific time intervals.

A number of plasma penicillin curves for patients receiving penicillin therapy only have been obtained under precisely standardized conditions. The samples were collected during the clinical evaluation of a compound that inhibits penicillin excretion.^{1, 2} Intramuscular injections were given at three-hour intervals, and penicillin doses ranged from 50,000 to 500,000 units per injection. The information that was accumulated has been of considerable value in predicting

* Received for publication, January 31, 1948.

the probable range of plasma penicillin concentrations at a given time following a specified dose, and in estimating the dosage necessary to attain a desired range of penicillin plasma concentrations. We are, therefore, presenting these data in the belief that they will be of value to clinicians and laboratory workers who are interested in penicillin therapy.

METHODS

Commercial crystalline penicillin sodium was used in all cases. It was restored with saline or distilled water, and given intramuscularly every three hours to hospitalized patients who were receiving penicillin therapy. No fewer than two injections of the specified dose had been administered before blood samples were drawn, and these samples routinely were taken 15, 30, 60, 120 and 180 minutes after the penicillin was given. Schedules for each patient were drawn up in advance, and special care was taken to see that the injections and bleedings were carried out at the exact times designated.

The blood, mixed with citrate in the syringe, was expelled into graduated tubes and was then centrifuged. Blood and citrate volumes were recorded; the plasma was withdrawn by capillary pipet, transferred to individual vials and immediately frozen. These samples were transported to the laboratory where they were maintained in the frozen state until assayed. Aseptic procedures were used at all times during the handling of these materials, and penicillin assays were performed on the same day, or on the day following, the arrival of the plasma in the laboratory.

A modification of the Rammelkamp *in vitro* assay¹² was used to determine penicillin concentrations. A stock standard solution containing 20 units of penicillin per cc. of phosphate buffer (pH 6) was made from crystalline penicillin (1667 units per milligram) and was stored at icebox temperature for not more than four days, after which time it was replaced by a fresh solution. Buffered saline (pH 7.2) was used to make half-step rather than double dilutions of the plasma and the standard. The assay organism, a group A hemolytic streptococcus, usually was sensitive to 0.039 unit of penicillin per cc. of plasma. The tests were incubated 18 to 20 hours at 37 C., and endpoints were read as the lowest concentration of penicillin that caused inhibition of hemolysis. Units of penicillin per cc. of plasma were calculated, and the values were corrected for the amount of citrate added to the blood. When necessary, a tube was included in the test which contained twice the amount of undiluted plasma routinely used. In this way a concentration of 0.019 unit of penicillin per cc. of citrated plasma could be approximated. For purposes of arithmetical averaging, samples containing concentrations of penicillin that could not be detected by this test were listed as zero values.

RESULTS AND DISCUSSION

The average concentrations of penicillin per cc. of plasma occurring at different times after the intramuscular injection of various doses of penicillin are listed in

Table 1. Here, also, are shown the number and the range of assay values that were averaged to obtain any one figure. It will be seen that for several of the doses the plasma concentration at the thirty-minute point tended to be the same or slightly higher than that reported at fifteen minutes. This is merely a reflection of the fact that some patients had not reached their maximal plasma penicillin concentration at the time of the fifteen-minute bleeding, while others had attained or passed their highest point on the penicillin curve at this time. It is widely recognized that the rate of absorption of penicillin into the blood

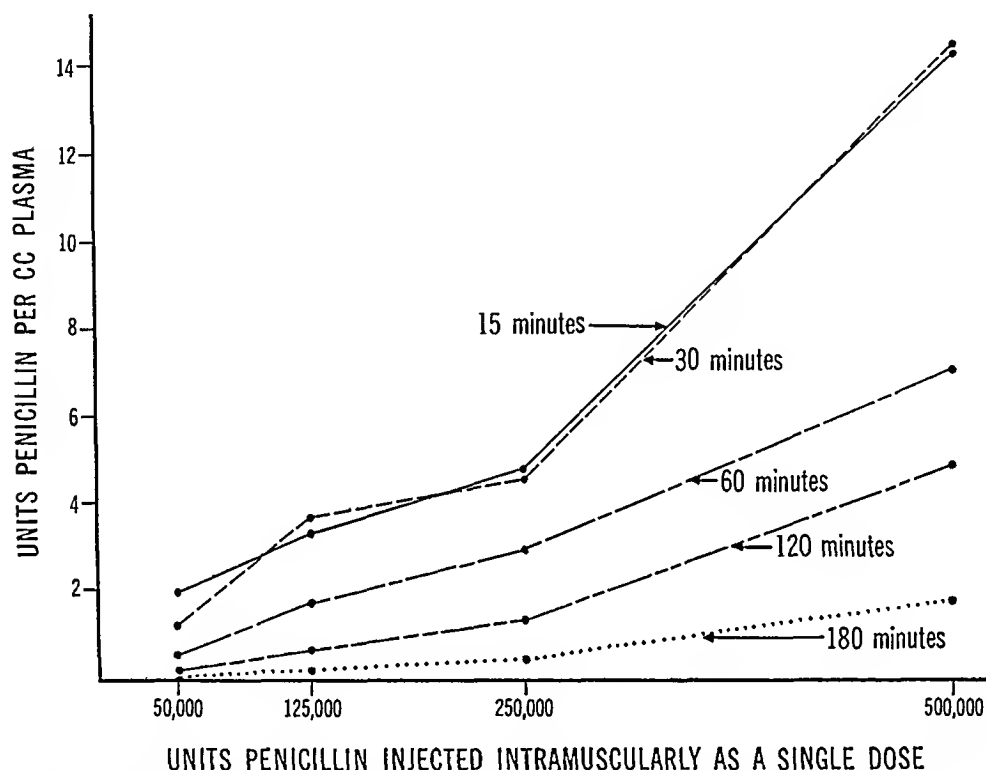


FIG. 1. Plasma penicillin concentrations at different times following intramuscular therapy

stream following intramuscular therapy will be influenced by conditions at the particular site of injection. Individual curves, therefore, may show a fifteen-minute point that is lower than, equal to, or higher than, the thirty-minute penicillin concentration.

Figure 1 shows a plotting of the average plasma penicillin concentrations according to the sampling time after the intramuscular injection of various doses of penicillin. With the data from Table 1 plotted in this way it is possible to estimate plasma penicillin concentrations that might be expected following the intramuscular administration of doses other than those reported here. In order to make proper application of this information it is necessary to know how much deviation from any of the average values plotted on this graph may be expected. Accordingly, each of the 104 individual assay figures used to calculate these 20

average points was examined to determine its variation from the appropriate group average. It will be recalled that the individual assay figures were calculated from the endpoint tubes demonstrating inhibition of hemolysis in a dilution series. If any one of the points plotted in Figure 1 is regarded as an endpoint tube in such a series, the deviation of the individual assays from this average value can be expressed in terms of the number of tubes in the dilution series separating the endpoint of the individual assays from the average endpoint. The maximum variation around each average may then be obtained by adding the tube variation of the highest and lowest penicillin values included in the group. When this was done, it was found that every one of the 104 assays performed in this study fell within the range of a ten-tube half-step dilution series set up around the average endpoint for the appropriate group. The overall tube

TABLE 1

AVERAGE PLASMA PENICILLIN CONCENTRATIONS FOLLOWING INTRAMUSCULAR INJECTION

UNITS PENICILLIN I.-M. Q. 3 HR.	NUMBER OF PATIENTS	UNITS PENICILLIN PER CC. PLASMA				
		Minutes after Injection of Penicillin				
		15	30	60	120	180
50,000	7	1.93 1.4-2.8(5)*	1.24 1.0-1.4(5)	0.52 .34-.69(7)	0.15 .09-.26(7)	0.02 0-.09(11)
125,000	6	3.42 2.0-4.2(7)	3.80 2.0-5.5(7)	1.79 1.0-2.8(6)	0.73 .25-1.4(7)	0.25 .13-.52(7)
250,000	3	4.84 4.2-5.5(2)	4.61 4.2-5.5(3)	3.06 2.7-3.7(3)	1.41 1.0-1.8(3)	0.52 .34-.69(2)
500,000	5	14.52 7.3-22(5)	14.74 6.0-33(5)	7.21 5.3-11(3)	5.06 1.8-8.3(4)	1.89 .33-4.5(5)

* Range of values averaged; number of assays given in parentheses.

variation for all the assays is shown in Figure 2. Such variation in individual response to intramuscular penicillin is rather small in view of the fact that for this study no selection of patients was made on the basis of age, weight, diseases under treatment, or renal function. All of these factors have been reported to influence the concentration of penicillin found in the blood stream following administration of penicillin.^{3, 13, 14} The possibility of such individual variation should be understood and taken into consideration by the clinician using penicillin.

It is also of importance to realize the extent of variation that is inherent in the type of assay used in the laboratory. To illustrate the range of values represented by an assay figure obtained from a half-step dilution test, a hypothetical plasma penicillin curve ranging from 12 to 1.5 units has been drawn in Figure 3. For purposes of demonstration it will be assumed that all of the plasma samples assayed for this curve were set up in identical series of 10 dilution tubes each and

that these dilutions were such as to allow readings of from 0.75 through 12 units per cc. of test material. It will be seen by reference to Figure 3 that the endpoint for the fifteen-minute sample of blood was read at the ninth tube of the series and, therefore, was plotted as containing 12 units of penicillin per cc. of test material. Actually, however, this plasma may have contained any unitage covered by the range between the ninth and the tenth tube (12 to 16 units per cc.). The thirty-minute blood sample did not contain 12 units per cc. because its end-

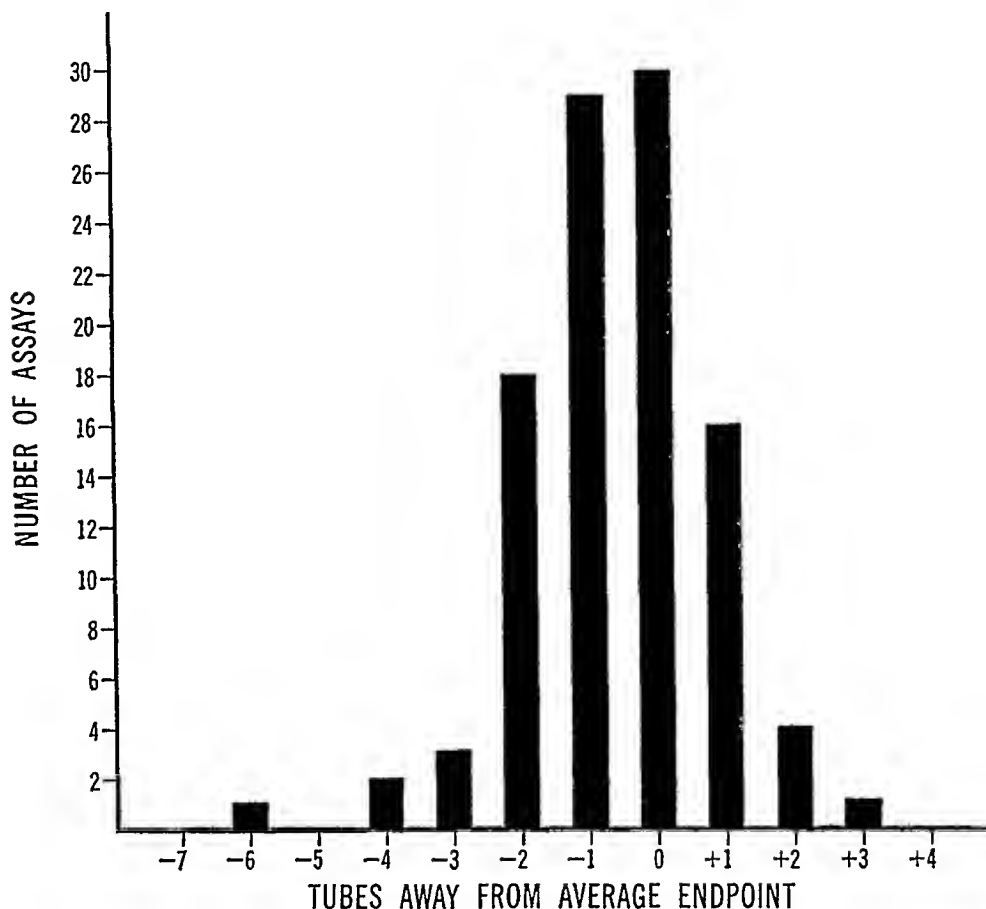


FIG. 2. Tube range of individual assays around average endpoints

point was the eighth and not the ninth tube. Because of the unitage differences between these endpoint tubes, however, the plotted point on the hypothetical curve represents any amount of penicillin between 8 and 12 units per cc. In Figure 3 the area between the reported endpoint tube and the next highest tube in the series has been shaded to indicate the possible range in units of the actual values of the reported assays.

It can be anticipated, then, that reports of higher values obtained by serial dilution assays may show rather wide apparent differences that actually are within the variations of the assay procedure. A reported plasma concentration of 30 units per cc., for example, should be interpreted as a value between 30 units

and 45 units, if a half-step dilution assay was used. Conversely, assay values in the hundredths or tenths of a unit are interpreted within a much smaller range in units although the percentage variation is similar.

The percentage difference between tubes in the half-step dilution test also must be considered by the laboratory worker who sets up the assay. Although each submitted sample must be treated as an "unknown", an estimation of the probable penicillin concentration may be made on the basis of past experience. From the known sensitivity of the assay organism and the anticipated penicillin

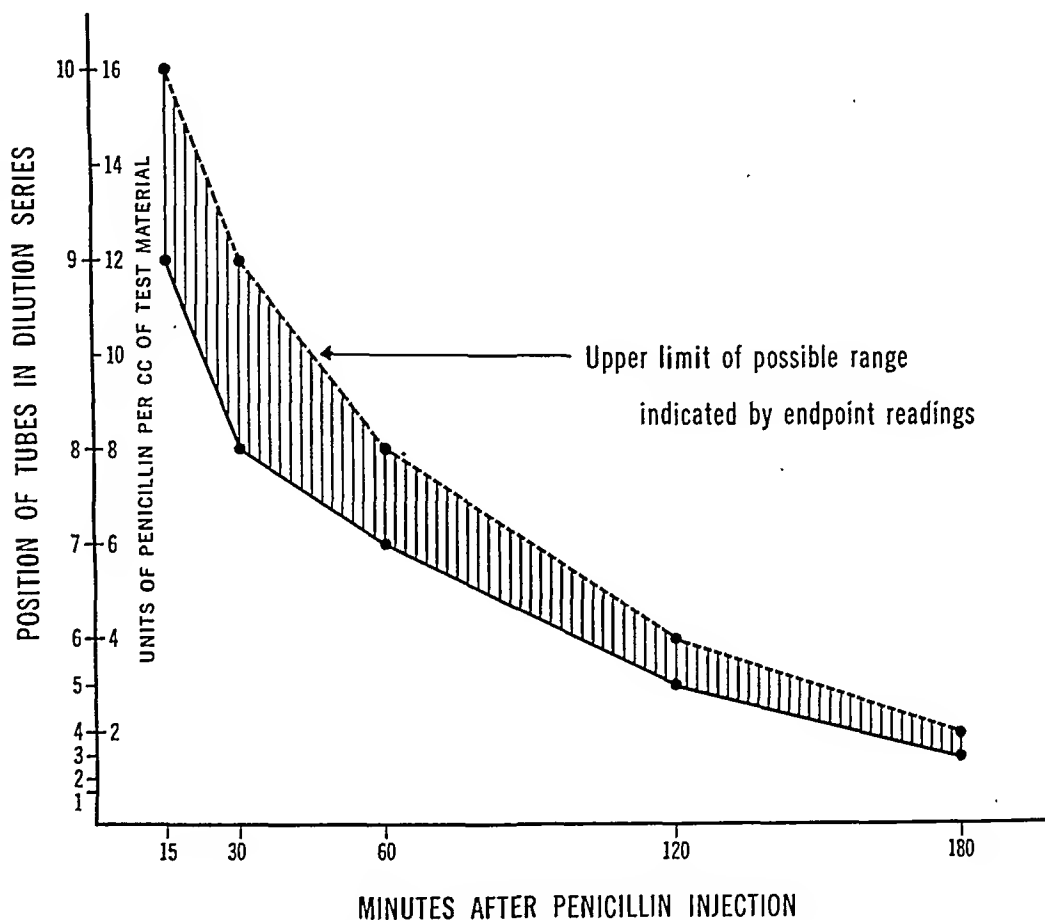


FIG. 3. Plasma penicillin curve plotted from endpoint readings and showing possible range of reported values

concentration in the plasma, the approximate plasma dilution necessary to reach an endpoint may be calculated. When this is a relatively high dilution it should be realized that, to obtain the greatest flexibility of a 10-tube half-step series, the dilutions should be so arranged that the expected endpoint will fall in the seventh tube. If this is done, and the penicillin concentration has been overestimated, it is possible to assay as little as 12.5 per cent (one-eighth) of the predicted concentration should the endpoint occur in the first tube of the series. If, on the other hand, the blood sample contained more than the expected amount of penicillin and the endpoint were read in the ninth tube, this would represent twice, or 200 per cent of the anticipated concentration. By reference to Figure

3 it may be seen that, had an expected endpoint been placed in the fifth tube (here calculated to 3 units per cc.), the lowest possible reading would have been 0.75 unit per cc. or only 25 per cent (one-fourth) of the expected 3 units, and the highest possible endpoint reading in this series would have been 12 units or four times the anticipated value.

An appreciation of the possibilities and limitations of the assay procedure used not only will be of value to the laboratory worker who sets up the test, but also such information is indispensable to the clinician who interprets the results. The variations in plasma concentration curves in different patients or in differing dosage schedules should be considered, not in terms of actual units, but in terms of percentage differences. For example, although a 10 unit difference exists in each case between comparable plasma samples reported to contain 30 and 40 units of penicillin per cc. and between comparable samples reported to contain 1 and 11 units per cc., the variation in the first set of plasmas would not be considered to have the same significance as it has in the second set.

We believe that these facts concerning the interpretation of data, together with the assay results reported here, will be of value to the clinician who is interested in the use of penicillin administered intramuscularly. The therapeutic plasma penicillin concentration desired for a patient may be determined by a consideration of the penicillin sensitivity and the known or suspected localization of the infecting organism in the patient's body. If the organism is quite sensitive to penicillin but the foci of infection are well protected from direct contact with the blood stream, the therapeutic plasma penicillin concentration will of necessity have to be greater than would be required were the infecting organism more directly exposed to the antibiotic agent present in the body fluids. The probable variation from the average in response to a given intramuscular injection of penicillin must be judged in relation to the age, weight and particularly the renal function of the individual patient. With these considerations in mind, the average data presented in Table 1 and Figure 1 may be useful in estimating a probable effective therapeutic regime, and a critical evaluation of laboratory reports will determine whether the desired plasma penicillin concentrations have been attained.

SUMMARY

Hospitalized patients were treated with 50,000, 125,000, 250,000, or 500,000 units of penicillin given intramuscularly every three hours. Blood samples for penicillin assay were taken 15, 30, 60, 120 and 180 minutes after the administering of penicillin. Average plasma penicillin concentration curves are presented to show the approximate plasma concentrations that may be anticipated following these doses of the antibiotic agent. The variations to be expected within the limits of a modified Rammelkamp type of assay and the application of these data to clinical practice are discussed.

Acknowledgment. The authors wish to express their appreciation to Elizabeth E. Hughes, B.A., for her excellent technical assistance.

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THE EFFECT OF VARIATIONS IN THE CONCENTRATION OF NON-PROTEIN CONSTITUENTS OF SERUM ON THE CORRELATION BETWEEN THE SPECIFIC GRAVITY AND THE PROTEIN CONTENT*

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The correlation between the specific gravity of serum and the protein content appears to be limited mainly, if not solely, by the extent to which variations in the concentration of the nonprotein constituents may alter the specific gravity. Since the reliability of the widely-used specific gravity methods for the estimation of serum protein is determined by the degree of this correlation, it seemed of interest to study the effects produced as a result of such variations.

While some investigators^{6, 7, 12, 19} have found a close correspondence between the total protein and the specific gravity, contrary results have been reported by others. Thus, Looney¹⁰ obtained a correlation coefficient of only 0.18 on serums from normal persons, and of 0.56 on specimens taken from a group of patients during diathermy. A similar low correlation between the two variables was observed by Cole *et al.*,^{3, 4} using rabbits in gravity shock, the divergencies being greater in animals not fasted than in animals from which food had been withheld for twenty-four hours prior to the production of shock. Recently, Berg and Perkins² reported a long series of parallel determinations on laboratory specimens by turbidimetric and specific gravity methods showing differences as great as 48 per cent with an average difference of 16 per cent, from which it was concluded that the latter method must be subject to errors of this magnitude. The lack of relationship noted in these studies was attributed by the different investigators to alterations in the concentrations of such nonprotein components as salt, fat, glucose and nonprotein nitrogen. Adams and Ballou¹ concluded that the correlation is too low to permit reliable estimation of serum protein by the specific gravity method, but were unable to find a satisfactory explanation for their failure to obtain better results. Previously, Zozaya²¹ had found a correlation coefficient of 0.28 when working with plasma from persons with normal, or nearly normal, protein levels. This author believed that his poor results could be explained on the basis of the presence of varying proportions of the different protein fractions in his samples, a conclusion which is not borne out, however, by the work of Nugent and Towle¹⁴ and Treffers¹⁸, who found albumin and globulin to exert equivalent effects upon the specific gravity.

The total contribution made to the specific gravity of normal serum by all the composite nonprotein substances may be estimated from any one of a number of empirical equations which have been developed for converting specific gravity

* This work was aided by grants from the Alumni Research Foundation of the College of Medical Evangelists. Received for publication, January 27, 1948.

values into protein concentrations. For example, in the equation of Moore and Van Slyke¹² as revised by Phillips *et al.*,¹⁶

$$P = 360 (G - 1.007)$$

where P is the concentration of protein in Gm. per 100 ml. and G is the observed specific gravity, it is evident that the constant 1.007 represents the specific gravity of serum with protein eliminated, and that 0.007, therefore, equals the total specific gravity contribution of the nonprotein constituents. Furthermore, if 1.0264 is taken as the specific gravity of normal serum, it follows that the non-protein fraction accounts for about 25 per cent of the difference between the specific gravities of serum and water, while the remaining 75 per cent is due to protein. As will be shown, more than four-fifths of the 25 per cent is caused by

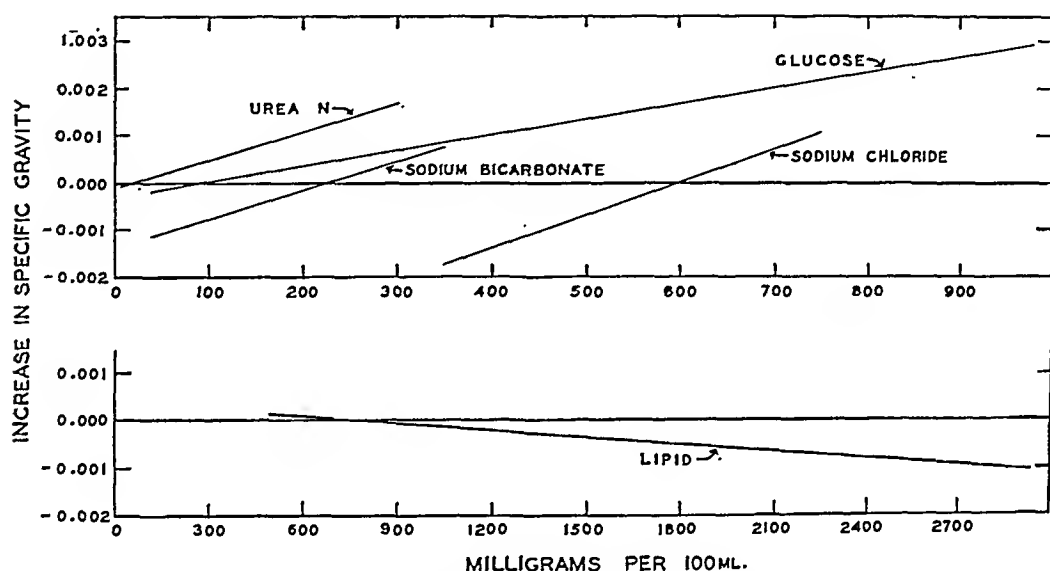


FIG. 1. Graphs showing the effect of variations in the concentration of nonprotein constituents on the specific gravity of serum. Each graph is so drawn as to intersect the horizontal axis at normal concentration.

the combined influence of chlorides, bicarbonates and glucose. In certain pathologic cases, urea and lipids also exert relatively large effects.

In the present work, a study was made of the effects produced on the specific gravity of serum and, hence, on the outcome of specific gravity determinations of protein, by variations in these quantitatively important constituents.

MATERIALS AND METHODS

Graded amounts of glucose, urea, sodium chloride, sodium bicarbonate and lipids were added to separate portions of pooled human serum, and the specific gravities of the resulting mixtures were determined. In most instances a 2 ml. Nicol-type pycnometer was used. At times an indirect specific gravity method¹³ previously described was employed, but only after having been standardized by means of pycnometry. The lipid material was obtained from serum by extracting with alcohol and ether, evaporating the solvents on a steam bath and drying

the lipid residue in a desiccator. Emulsions of this residue in serum were prepared for measurement. While lipids added in this way are probably present in a state somewhat different from that of lipids occurring naturally in serum, they should have at least an approximately equivalent influence on the specific gravity.

In the second phase of this study, analyses were made on serums from patients with diseases in which abnormalities in protein or in important nonprotein constituents are as large as are likely to be encountered. Thirty cases were studied. These included persons suffering from uncontrolled diabetes, nephritis, nephrosis and liver cirrhosis.

Protein analyses were made by the macro-Kjeldahl determination of nitrogen, employing a three hour digestion in the presence of copper and selenium catalysts. The methods used for other constituents were: glucose, Folin and Wu;⁵ urea, Karr;⁸ chloride, Whitehorn;²⁰ bicarbonate, Peters and Van Slyke;¹⁵ cholesterol, Sackett;¹⁷ total lipids, Meigs and Marsh.¹¹

RESULTS

The graphs shown in Figure 1 represent the effect of the varying levels of non-protein constituents on the specific gravity of serum. They were constructed by plotting specific gravity against the concentrations of the added substances, and then extrapolating the values of concentration to cover the usual normal and abnormal ranges. As could be expected, these graphs are nearly identical with the specific gravity curves for solutions of the same solutes in pure water.

In order to change the specific gravity by an amount equivalent to 0.25 Gm. of protein per 100 ml., the concentration of sodium chloride must be increased or decreased by about 100 mg. per 100 ml.; sodium bicarbonate, 115 mg.; glucose, 210 mg.; urea nitrogen, 120 mg.; and total lipids, 1400 mg. The specific gravity increments for each 100 mg. of constituent per 100 ml. of serum, together with their protein equivalents, as computed by the formula of Phillips *et al.*,¹⁶ are given in Table 1. It will be noted that the lipids extracted from nephrotic serum produced a smaller effect than lipids obtained from pooled normal specimens. This difference is presumably caused by the greater proportion of cholesterol in the nephrotic serum. Free cholesterol should have little influence, since its density approximates that of serum.

Figure 2 depicts the specific gravity patterns of typical abnormal serums as calculated from chemical analyses and from the corresponding specific gravity increments given in Table 1. In constructing the diagrams, it was assumed that each serum component makes its characteristic contribution to the total specific gravity. For simplicity, all chlorides and bicarbonates were regarded as being present in combination with sodium ion. This ion comprises about nine-tenths of the total base. Furthermore, the next most abundant ion, potassium, yields salts which have nearly the same specific gravities in solution as the corresponding salts of sodium. The specific gravity increment due to the undetermined constituents was found indirectly in each case by subtracting the sum of the known increments from the specific gravity of the serum sample. Being thus obtained

by difference, this quantity is necessarily subject to a relatively large percentage error. It should be pointed out, also, that in a chemical system as complicated

TABLE 1
EFFECT OF INCREASING THE CONCENTRATION OF SERUM CONSTITUENTS ON SPECIFIC GRAVITY

CONSTITUENT	SPECIFIC GRAVITY INCREMENT*	EQUIVALENT PROTEIN CONCENTRATION INCREMENT†
Protein.....	0.000278	0.10
Sodium chloride.....	0.00070	0.25
Sodium bicarbonate.....	0.00060	0.22
Glucose.....	0.00033	0.12
Urea nitrogen.....	0.00058	0.21
Lipids, normal.....	0.000056‡	0.020‡
Lipids, nephrotic.....	0.000048‡	0.017‡

* Change in specific gravity for each 100 mg. of constituent per 100 ml. of serum.
† Grams of protein per 100 ml. of serum.
‡ Decrease.

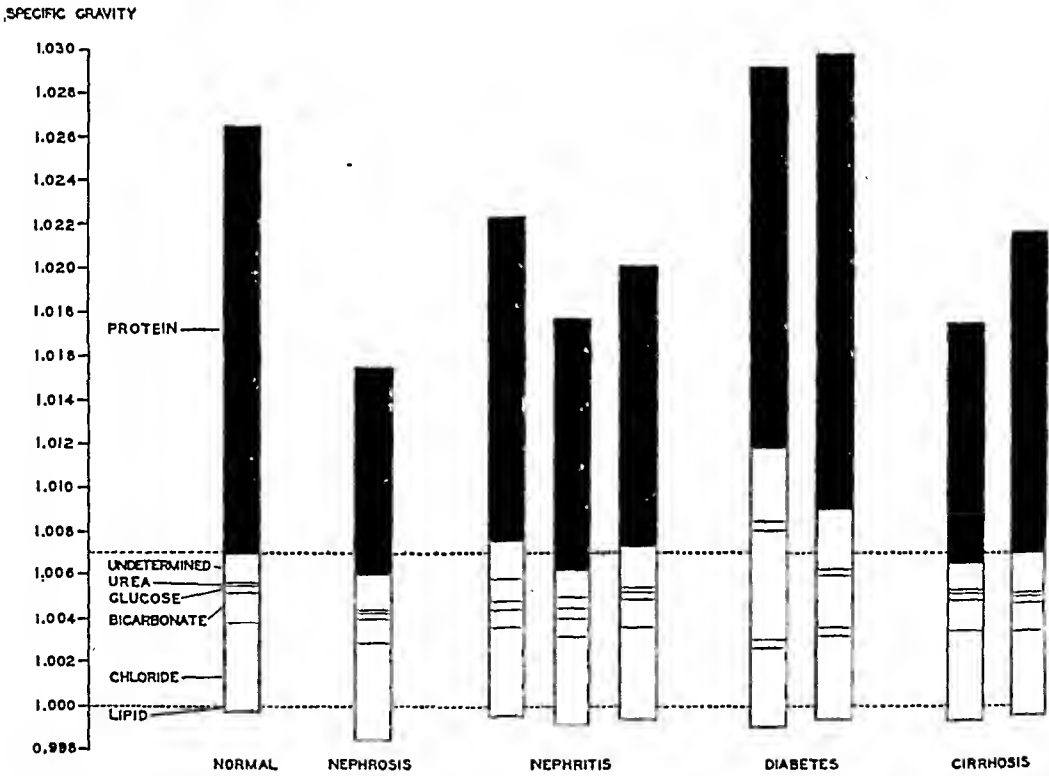


FIG. 2. Graphic representation of the specific gravity structure of normal and abnormal serums. Bases of columns coincide with the specific gravities of the serums with all solutes except lipids eliminated. The lowering of specific gravity caused by the presence of lipids is, therefore, represented by the distance which a column protrudes below the broken line at 1.000. The upper broken line indicates the specific gravity of normal serum with protein removed.

as serum there are many interactions, and the contributions of the component substances are, consequently, not strictly additive. For these reasons, the

specific gravity patterns are only approximate. They, nevertheless, provide a view of the relative importance of the main constituents in determining the specific gravity of serum, and indicate the order of magnitude of the specific gravity changes caused by departures from the normal levels of these constituents in the conditions studied.

The first column in Figure 2 represents serum with a normal composition. The second column depicts findings in serum from a nephrotic patient with a lipid content of 3100 mg. per 100 ml. The third, fourth and fifth columns represent serums from nephritic patients containing respectively 170, 95 and 40 mg. of urea nitrogen per 100 ml.; the sixth and seventh, diabetics with 1500 and 750 mg. of glucose per 100 ml.; and the eighth and ninth, cirrhotic individuals with no large abnormalities in nonprotein composition.

In the nephrotic type of renal disease the specific gravity appears to be lowered, as a rule, in nearly direct relation to the increase in lipids, although a rise in chloride without a corresponding fall in bicarbonate may lessen the effect of lipemia. The changes occurring in chronic nephritis are somewhat irregular, but in such cases any retention of urea tends to be compensated by loss of chloride and bicarbonate, and by an increase in lipids other than cholesterol. In diabetes the specific gravity is usually increased by an amount which almost parallels the elevation of the glucose level. At times, however, the influence of the extra glucose appears to be slightly diminished by lowered salt concentration and increased lipids. It will be observed that the effect of the undetermined constituents is here considerably greater. This may be attributed largely to the presence of ketone acids which become quantitatively important electrolytes in severe diabetes. The variations occurring in liver cirrhosis are apparently of insufficient magnitude to alter significantly the specific gravity.

The greatest variation from normal specific gravity attributable to abnormalities in nonprotein composition, encountered in these and similar cases which were studied, did not exceed the equivalent of 0.4 Gm. of protein per 100 ml., except in nephrosis with especially high serum lipid and in diabetes with a glucose concentration as great as four or five times the normal value.

From these results it may be concluded that the specific gravity method should, with the foregoing exceptions, be capable of yielding protein concentrations within a limit of error of this magnitude, *i. e.*, ± 0.4 Gm., provided, of course, that the effect of protein itself on specific gravity is invariable and is accurately known. It seems probable, therefore, that the larger discrepancies (1 to 2 per cent of protein) which have been reported as commonly occurring in the specific gravity-serum protein relationship, must in large measure be accounted for by variations in the reliability of the analytic methods employed.

SUMMARY

1. The extent to which variations in the quantitatively important nonprotein constituents affect the specific gravity of serum was studied.
2. To change the specific gravity by an amount equivalent to 0.25 Gm. of protein per 100 ml., the concentration of sodium chloride must vary 100 mg. per

100 ml.; sodium bicarbonate, 115 mg.; glucose, 210 mg.; urea nitrogen, 120 mg.; and total lipids, 1400 mg.

3. The bearing of these results on the specific gravity method for the determination of serum protein is discussed.

Acknowledgment. I am indebted to Miss Margaret Anderson, B.S., for assistance in this work.

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A SIMPLE METHOD OF DETERMINING NONPROTEIN NITROGEN, TOTAL PROTEIN AND ALBUMIN IN BLOOD SERUM SAMPLES BY USING CONWAY CELLS*

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In the course of another study it was necessary to determine proteins in many samples of human blood serum and ascitic fluid. Attempts to use the micro-Kjeldahl method were found inadequate because of the amount of time required to carry out a determination and the complexity of the distillation procedure. The Conway diffusion cell¹ was used earlier in this laboratory to determine the ammonia content of urine, and this method was adapted to the determination of serum proteins. The principle of the Conway diffusion method is based on the transfer of the volatile material to be determined by simple diffusion from the outer portion of the cell, where it is produced, to the central portion of the cell, where it is trapped and finally estimated. This study is a report of the comparative data obtained by using the micro-Kjeldahl and Conway diffusion techniques.

METHODS

Reagents:

Sulfuric acid, concentrated

Sulfuric acid, 0.01 normal

Sodium hydroxide, 50-60 per cent solution

Superoxal

Trichloroacetic acid, 10 per cent solution

Boric acid, 2 per cent solution

Methyl red-bromocresol green mixed indicator of Ma and Zuazaga.² (This reagent is prepared by mixing 11 parts of a 1 per cent alcoholic solution of bromocresol green with 2 parts of a 1 per cent alcoholic solution of methyl red.)

Sodium sulfate, 22.2 per cent solution

The micro-Kjeldahl distillations were carried out in an all-glass distillation of the Kemmer and Hallette type. The distillation technic was essentially that presented by Miller and Houghton.⁴

Determination of Nonprotein Nitrogen

One ml. of serum was added to a centrifuge tube containing 9 ml. of trichloroacetic acid and the mixture well shaken. It was allowed to stand for about five minutes and then centrifuged. Five ml. of the supernatant fluid was pipetted

*Received for publication, December 27, 1947.

into a large pyrex test tube (200 x 25 mm.) graduated at 12.5 ml. After adding 1 ml. of concentrated sulfuric acid, the solution was digested using a micro-Kjeldahl digestion rack. Addition of a few drops of superoxal was used to complete the digestion. After cooling, the digested sample was diluted to mark. A 5 ml. aliquot of this solution was used for the determination, the following procedure being used for all the diffusion studies. The sample of the digest was pipetted into the outer chamber of a Conway cell. The center chamber contained from 2 to 3 ml. of boric acid solution plus a drop of the mixed indicator. The glass cover of the cell was greased on one side with petrolatum and placed greased side down on the cell in such a manner that all but a small portion of the outer chamber was covered. Through this opening from 2 to 3 ml. of strong sodium hydroxide was added rapidly from a wide tipped pipet. Care was taken that the contents of the pipet were not blown out since some of the alkali might spatter into the center chamber and thus invalidate the results. Immediately after the addition of the alkali, the glass cover was slid over, rapidly sealing the entire cell; it is essential that an airtight contact be made between the cell and the covering plate. The acid digest and alkali were mixed in the outer chamber by gentle rocking of the entire cell. The cell was allowed to stand for at least five hours at room temperature to allow complete diffusion of the liberated ammonia from the outer chamber into the inner chamber.

The amount of ammonia which diffused into the center chamber and combined with the boric acid was determined by titration with 0.01 N sulfuric acid. The amount of ammonia present in the aliquots used for the determinations was found to diffuse quantitatively from the outer chamber to the inner chamber in five hours at room temperature. Also, since the original sample was completely digested with sulfuric acid, no compounds such as urea, were present, which might break down slowly to yield ammonia. Increase in diffusion time to as long as forty-eight hours did not affect the determination.

Determination of Total Serum Protein and Serum Albumin

One ml. of serum was digested in a large pyrex test tube (200 x 25 mm.) calibrated at 50 ml. capacity. The digestion was carried out by adding one ml. of sulfuric acid, and the final clearing of the solution was accomplished by adding a few drops of superoxal. After digestion, the sample was diluted to the 50 ml. mark. One ml. of this solution was used for the determination of the total protein nitrogen. The determination of the protein nitrogen was the same as that used for the determination of the nonprotein nitrogen, except that a 1 ml. sample rather than a 5 ml. sample was used.

Determination of serum albumin was carried out according to the method proposed by Howe.² One ml. of serum was added to 29 ml. of 22.2 per cent sodium sulfate and was allowed to stand three hours in an incubator at 37 C. The globulin precipitate was filtered off, and the albumin nitrogen was estimated by using 5 ml. of the filtrate. The digestion and determination of the albumin nitrogen were the same as those used for the determination of the nonprotein nitrogen.

Calculations:

Nonprotein nitrogen mg. per cent	= Titration \times 70
Total protein Gm. per cent	= (Titration \times 700 - NPN) \times 0.00625
Albumin Gm. per cent	= Titration \times 3.675
Globulin Gm. per cent	= Total protein - albumin

RESULTS AND DISCUSSION

The values obtained by using the micro-Kjeldahl and Conway diffusion methods for determining nitrogen are presented in Table 1. A comparison of these shows a satisfactory agreement between the two methods. The accuracy of the two methods appears to be approximately the same, but the precision of

TABLE 1

COMPARISON OF THE KJELDAHL AND CONWAY METHOD FOR THE DETERMINATION OF PROTEIN NITROGEN

The nonprotein nitrogen values (mg. per 100 ml.) have been subtracted from those of the total protein

BLOOD SAMPLE	MICRO-KJELDAHL METHOD					CONWAY METHOD				
	NPN	Total Protein	Albumin	Globulin	A/G Ratio	NPN	Total Protein	Albumin	Globulin	A/G Ratio
	Mg.	Gm.	Gm.	Gm.		Mg.	Gm.	Gm.	Gm.	
1	32.4	5.98	3.22	2.76	1.17	31.1	6.02	3.22	2.80	1.15
2	37.5	6.95	3.96	2.99	1.32	30.8	6.95	4.01	2.94	1.36
3	20.0	7.05	4.68	2.37	1.97	22.3	7.10	4.72	2.38	1.98
4	86.4	5.72	3.98	1.74	2.28	86.4	5.80	3.98	1.72	2.31
5	30.5	6.70	3.80	2.90	1.32	30.5	6.70	3.78	2.92	1.30
6	21.6	6.35	3.18	3.17	1.00	22.3	6.35	3.22	3.13	1.03
7	35.5	6.95	3.73	3.22	1.16	36.0	6.95	3.78	3.17	1.19
8	21.0	6.95	4.21	2.74	1.53	21.0	7.00	4.10	2.90	1.42

the Conway diffusion method is greater than the micro-Kjeldahl distillation, particularly in the determination of the nonprotein nitrogen.

The glass Conway cells were used in all diffusion determinations in this study, but in the determination of total protein the porcelain cells may be used. They are not as convenient as the glass cells for the determination of the nonprotein nitrogen or albumin since the volume of the outer cell approaches the volume of the solutions added and spillage of the contents of the outer chamber into the inner chamber occurred frequently, thus invalidating the determination.

The Conway diffusion methods outlined here have been used for the determination of serum proteins in our laboratory for about a year by different technicians without experiencing difficulty. Since only three quantitative measurements are required, measuring of the serum sample, pipetting of an aliquot of the digested sample into the Conway cell and titration of the boric acid solution with 0.01 N sulfuric acid, this type of analysis reduces errors of manipulation

to a minimum. In addition, after setting up the cell, no manipulations or observations are required until the titrations are started. If it is not convenient to open the cells at the end of the five hour period, the period of standing may be increased to twenty-four or forty-eight hours without any effect on the determination. Thus, by using these methods, it is possible to do many serum protein analyses per day without seriously interrupting general laboratory routine. In this study, the Conway diffusion methods were applied to the determination of total protein and albumin using the Howe salt fraction methods, but it could be applied to any protein determination where the micro-Kjeldahl method is used for nitrogen determination.

SUMMARY

A method is presented which permits the determination of the nonprotein nitrogen, total protein, albumin and globulin fractions of human serum by use of the Conway diffusion cell. This method is relatively simple and does not require the constant attention of the technician. Agreement was obtained in comparison of this method with the micro-Kjeldahl distillation on 8 samples of human serum.

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APPLICATION OF THE WEICHELBAUM BIURET REAGENT TO THE DETERMINATION OF SPINAL FLUID PROTEIN*

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The method of Weichselbaum¹ for the determination of serum proteins has been checked in the writer's laboratory against the micro-Kjeldahl method and found to yield reproducible, accurate results. Because of the technical simplicity, accuracy and sensitivity of the method, it has been possible to adopt it into a satisfactory procedure for the determination of spinal fluid proteins.

APPARATUS AND REAGENTS

Optical densities were measured with a Coleman model 6 A spectrophotometer at 555 mu. using number 308 cuvetts. The more sensitive biuret reagent of Weichselbaum of the following composition (per liter of solution) was used:

9.0 grams sodium potassium tartrate
3.0 grams $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$
5.0 grams KI
0.2 N NaOH (carbonate-free)

The Rochelle salt is dissolved in approximately 400 ml. of 0.2 N NaOH to which the copper sulfate is added and dissolved with stirring. The potassium iodide is then dissolved and the solution diluted to 1000 ml.

Protein standards were prepared from pooled serum analyzed by the micro-Kjeldahl method. Dilutions were made to represent 15, 30, 60 and 120 mg. of protein per 100 ml., and an optical density-concentration graph prepared. The graph is a straight line within the range of the above concentrations.

METHOD

One ml. of spinal fluid and 1 ml. of biuret reagent are mixed in a Kahn tube. A blank tube is prepared similarly with 1 ml. of biuret reagent. Both tubes are thoroughly mixed and placed in a 37 C. water bath for thirty minutes. At the end of this time, the solutions are transferred at once to cuvetts and read immediately in the spectrophotometer. The concentration is calculated by multiplying the optical density (O.D.) by the factor K. K may be calculated after the preparation of the optical density-concentration graph from the formula $K = C/D$, where C is the concentration of a known sample and D is the optical density.

Suitable dilutions of spinal fluids containing large amounts of protein are made so that the range will fall within 150 mg. per 100 ml. The final result is multiplied by the proper dilution factor. To estimate the necessary dilutions, the following qualitative chart is used:

Globulin 1 plus = 50 to 100 mg. protein per 100 ml.
Globulin 2 plus = 100 to 300 mg. protein per 100 ml.
Globulin 3 plus = 300 to 500 mg. protein per 100 ml.
Globulin 4 plus = over 500 mg. protein per 100 ml.

* Received for publication, January 16, 1948.

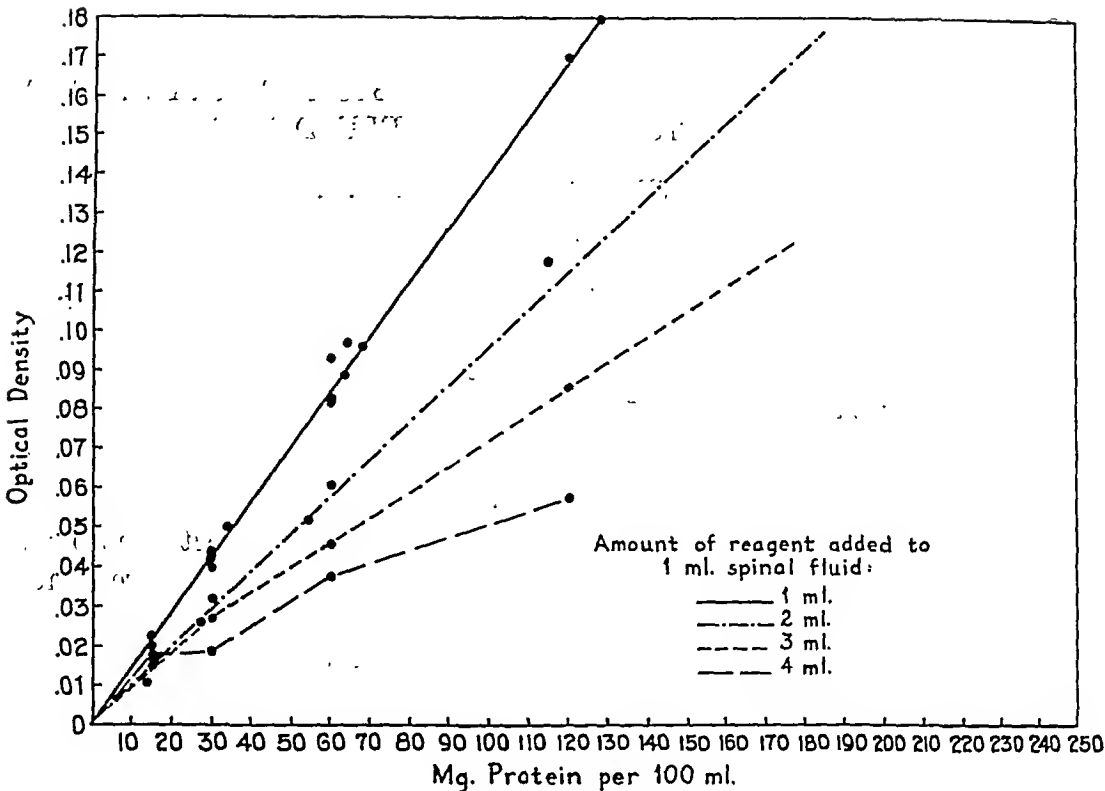


FIG. 1

TABLE 1
COMPARISON OF RESULTS OF TOTAL SERUM BY MICRO-KJELDAHL AND SPINAL FLUID METHODS

SERUM	TOTAL PROTEIN (GM. PER 100 ML.)	TOTAL PROTEIN CALCULATED FROM SPINAL FLUID METHOD (GM. PER 100 ML.)	PER CENT ERROR
1	6.23	6.20	-0.04
2	6.60	6.40	-3.0
3	6.60	6.65	+0.07
4	4.00	4.10	+2.5
5	5.50	5.50	0.0
6	6.30	6.70	+6.0
7	6.25	6.32	+1.0
8	6.00	6.20	+3.0
9	6.60	6.50	-1.0
10	6.45	6.60	+2.0
Average.....			1.8

* These determinations were run under ordinary commercial laboratory conditions. The measuring pipets used were both the Folin-Ostwald type and the standard 1 ml. serological. Dilutions were made in volumetric flasks.

DISCUSSION

The effect of temperatures of 37 C. and 56 C. on the reaction was compared. These temperatures were selected as being those commonly in use in laboratory water baths. Weichselbaum¹ states that an increase in temperature increases

the value of the optical density. We found this to be true, but selected the 37 C. temperature after it was noted that some spinal fluids showed reduction of biuret solution at the higher temperature.

An attempt was likewise made to select the optimum ratio of spinal fluid and biuret reagent. Figure 1 shows the optical density-concentration graphs using various amounts of biuret reagent with one ml. of spinal fluid. This quantity of spinal fluid was selected since most specimens received by us are inadequate for the consistent use of a larger quantity.

As a check on the accuracy of this micro-method, 1:100 dilutions were made of a series of previously determined serum proteins. One ml. quantities were treated in the same fashion as spinal fluid. The milligrams protein per 100 ml. were read from the previously constructed optical density-concentration graph, and the total serum protein was calculated by multiplying this figure by 100. The table below shows the results of this comparison.

SUMMARY

A rapid, accurate method is described for the determination of spinal fluid protein.

REFERENCE

1. WEICHSELBAUM, T. E.: An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. *Am. J. Clin. Path., Tech. Bull.*, **10**: 40-49, 1946.

A SIMPLE IMPROVEMENT OF THE COMMON SPRING LANCET TO REDUCE THE PAIN OF FINGER PUNCTURES*

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The common spring lancet for finger puncture has the disadvantage that the blade remains extended after the puncture is made. This doubtless increases the pain which, however slight, adds to the difficulty of obtaining blood samples from volunteers, particularly small children. A modification of the ordinary automatic blood lancet is described in which the blade springs back flush with the surface of the skin a fraction of a second after penetration. Although I have been informed that lancets are available which have a spring return on the blade, they are not in common use and none could be supplied through the larger laboratory supply houses which were contacted.

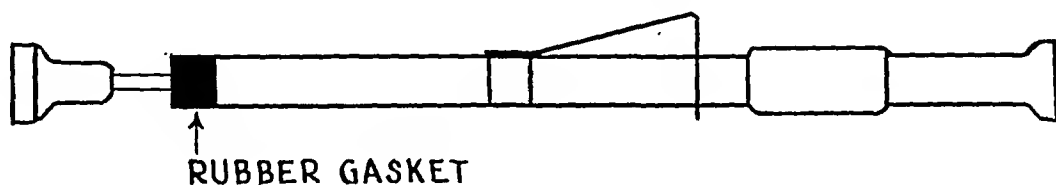


FIG. 1

A common spring lancet can easily be converted to a "blade return lancet" by placing a rubber gasket between the stopping knob and the end of the lancet body. Such a gasket is easily made by cutting the end from a Size 1A-66F (West Company, Phoenixville, Pennsylvania) vaccine bottle stopper or by cutting a piece of rubber tubing of proper size and elasticity. It is placed on the lancet by simply unscrewing the knob on the end of the lancet shaft, slipping the gasket on, and tightening the knob. The gasket should be 4 to 5 mm. thick for most purposes, although it may be varied somewhat depending upon the tension of the driving spring in the particular lancet and on the depth of the cut desired. The gasket, in addition, muffles the "click" which, in itself, may frighten infants and children. The lancet, so modified, was reported to be nearly painless by those subjects who were tested.

* Received for publication, December 22, 1947.

FIRST REPORT OF THE COMMITTEE FOR CLARIFICATION OF THE NOMENCLATURE OF CELLS AND DISEASES OF THE BLOOD AND BLOOD-FORMING ORGANS

A committee for clarification of the nomenclature of cells and diseases of the blood and blood-forming organs held its first meeting in Chicago at the Drake Hotel and Northwestern University Medical School, October 25-26, 1947. The committee was sponsored by the American Society of Clinical Pathologists and the American Medical Association. Additional sponsorship by any other interested groups will be welcomed.

Those present were representative of most geographic areas in the United States and most points of view in the field of hematology, and included clinical laboratory directors, editors of medical journals and hematologists. The following were present: Dr. Edwin E. Osgood, Chairman, Portland, Oregon; Dr. Howard L. Alt, Chicago; Dr. Lawrence Berman, Detroit; Dr. William Dameshek, Boston; Dr. Hal Downey, Minneapolis; Dr. Alvin G. Foord, Pasadena; Dr. Claude E. Forkner, New York City; Dr. A. S. Giordano, South Bend, Indiana; Dr. S. E. Gould, Eloise, Michigan; Dr. Byron E. Hall, Rochester, Minnesota; Dr. L. R. Limarzi, Chicago; Dr. Stacy Mettier, San Francisco; Dr. J. J. Moore, Chicago; and Dr. Nathan Rosenthal, New York City.

At this first meeting the following recommendations were adopted by those present and the report in its present form has been approved by all those whose names are appended below.

Clarification and definition of terms is urgently needed for the sake of a common understanding in clinical usage and in teaching of medical students and technicians. The choice of a preferred term, it was agreed, should not be based merely on historical priority or common usage but, in general, should represent the simplest, clearest and most descriptive term. Eponyms and new terms should be avoided, wherever possible, without sacrifice of clarity. An effort should be made to attain consistency between related terms.

The recommendations of the committee are to be tentative until they have been publicized and acted on by interested groups. The committee will welcome any suggestions and criticisms. It is planned that a committee should re-examine the terms periodically, perhaps every five or ten years.

The various series of cells were considered. It was recommended that in Table 1 the term listed at the left replace all terms listed at the right in referring to cells of a particular series or to a disease affecting any cell of that series.

No changes were suggested in the criteria in current use for determining the series to which a cell belongs. It is hoped, however, that the advances now being made in histochemistry will contribute more clearcut criteria than are available at present.

It is recommended that the term *leukocyte* be considered synonymous with

white blood corpuscle and include all white cells of the blood and their precursors in the blood-forming organs. Its use should not be limited to cells of the granulocytic series, excluding cells of the lymphocytic, monocytic or plasmacytic series. This and other words derived from the same root should be spelled with a *k* and not a *c*, e.g., leukocyte, leukemia, not leucocyte or leucemia.

It is recommended that the descriptive terms for granules, *neutrophil*, *eosinophil*, *basophil*, and *azurophil* be spelled as indicated without a final *e*.

It is suggested that the name of the most undifferentiated of the cells of each series carry the suffix *-blast*, the second stage the prefix *pro-* and, except in the granulocytic series, all cells that are more mature than the *-blast* stage have names with the suffix *-cyte*. The name for the fourth stage in the granulocytic and erythrocytic series is to have the prefix *meta-*. The terms *blast cells* and *pro cells* may be used to replace other terms for these stages of development when speaking of the stage of development as a whole or when the series to which the cells belong has not been identified.

It is recognized that the *blast cells* of each series are morphologically very similar, all having fine nuclear chromatin structure, usually demonstrable nucleoli, and basophilic cytoplasm, with or without azurophil granules, so the prefix to be used will, in many instances, depend on the identification of the *pro-* stage associated with them.

Fine chromatin structure is defined as having the nuclear appearance of a background of homogeneous lighter-staining parachromatin, overlaid by a darker-staining lace-net meshwork or finely stippled pattern of basichromatin, with no aggregation of the basichromatin into even a single clump of appreciable size staining darker than any other areas in the nucleus.

A *nucleolus* is defined as a homogeneous blue-staining area within the nucleus of a cell, which stains more like the cytoplasm than does any other part of the nucleus.

The term *azurophil* should be applied to the granules seen typically in the cytoplasm of cells of the lymphocytic and monocytic series and the progranulocyte stage of the granulocytic series. The term *azurophil* is recommended, and not *azure*, in describing these granules, since the term refers to an affinity for a particular dye and not to the color of the granules. These granules may be present or absent in any cell of the lymphocytic series and when present are usually coarse and in clumps. They are usually present in all cells of the monocytic series, including the monoblast. In the monocytic series they are usually fine, diffusely and uniformly scattered through the cytoplasm. If not seen in the monocyte or promonocyte, it usually indicates a faulty stain or poor visual definition in the microscope. These granules may be present or absent in any cell of the granulocytic series. They are rarely seen beyond the myelocyte stage except in disease. They are occasionally present in the cytoplasm of cells of the plasmacytic and erythrocytic series, and constantly present in the cells beyond the blast stage in the thrombocytic series where they tend to be fine and few in the early stages and numerous and often clumped in the more mature stages.

It is recognized that in each cell series there is a continuous development from

the most undifferentiated to the most differentiated stage, that an infinite number of subdivisions are possible, and that any subdivision is arbitrary. The committee recommended the use of the minimum number of subdivisions which will provide essential information for diagnostic and prognostic purposes and defined the lines of division between these stages as clearly as possible, basing these divisions on a single easily identifiable feature. As far as possible, the feature selected to differentiate the different stages of development is one which could be recognized in either stained or supravital preparations, but it is realized that at present the majority of such decisions will be based on smears stained with Wright's stain or with one of the other Romanowsky stains. Even with these definitions, cells will be encountered where decision is difficult, in which

TABLE 1

RECOMMENDED TERMS AND TERMS TO BE AVOIDED WHEN REFERRING TO CELLS
OF A PARTICULAR SERIES OR TO A DISEASE AFFECTING ANY CELL OF THAT
SERIES

TERM TO BE USED	TERMS TO BE AVOIDED
Lymphocytic	Lymphoid, lymphatic, lymphogenous, lymphocyte, mononuclear
Granulocytic	Myeloid, myelogenous, myelocyte, myelocytic, granulocyte, leukocyte, leukocytic, leucocyte, leucocytic
Monocytic	Monocytoid, monocytogenous, mononuclear, monocyte
Plasmacytic	Plasma cellular, plasmacytogenous, myeloma cell, plasmacyte
Erythrocytic	Erythroid, erythrocytoid, erythron, erythrocytogenous, erythrocyte
Thrombocytic	Megakaryocytic, platelet, thrombocyte

case it is suggested that the cell be arbitrarily placed in the more differentiated category.

Names were selected for each of the cells, which were acceptable to all members present and which, in the opinion of the committee, were least likely to be confusing.

The recommended terms and the terms to be avoided are listed in Table 2.

It is not the intention of the committee to imply from its recommendation of terms to be used that the origin of all these cells has been settled.

It is recognized that to ensure flexibility and for certain specialized purposes finer subdivisions may be necessary than those herein recommended. It is suggested that in such case no change be made in the term or definition of the recommended major divisions but that *clearly defined* qualifying adjectives be used for these further subdivisions. Should new knowledge indicate that another major cell division is needed the evidence for this need, together with the suggested term, should be submitted for consideration by a permanent body which it is hoped will develop out of this committee.

TABLE 2

RECOMMENDED TERMS AND TERMS TO BE AVOIDED WHEN REFERRING TO SPECIFIC CELLS OF THE BLOOD AND BLOOD-FORMING ORGANS

NAME OF SERIES	TERM TO BE USED	TERMS TO BE AVOIDED
Lymphocytic	Lymphoblast	Myeloblast, hemocytoblast, lymphoidocyte, stem cell, lymphocyte
	Prolymphocyte	Large lymphocyte, pathologic large lymphocyte, atypical leukocytoid lymphocyte, monocyte, immature lymphocyte
	Lymphocyte	Small, medium or large lymphocyte, normal lymphocyte, small, medium or large mononuclear
Monocytic	Monoblast	Myeloblast, hemocytoblast, lymphoidocyte, lymphocyte, stem cell, immature monocyte
	Promonocyte	Premonocyte, hemohistioblast, immature monocyte, Ferrata cell
	Monocyte	Large mononuclear, transitional, clasmatoocyte, endothelial leukocyte, histiocyte, resting wandering cell
Granulocytic	Myeloblast	Granuloblast, hemocytoblast, lymphoidocyte, lymphocyte, stem cell
	Progranulocyte	Promyelocyte II, leukoblast, myeloblast, premyelocyte, promyelocyte, progranulocyte A
	Myelocyte	Granulocyte, myelocyte B, non-filament, class I
	Metamyelocyte	Metagranulocyte, juvenile, myelocyte C, non-filament, class I
	Band cell	Staff cell, stab cell, non-filament, class I, rod nuclear, polymorphonuclear, stabkernige, rhabdocyte, non-segmented
	Segmented	Polymorphonuclear, filamented, class II, III, IV, or V, lobocyte
Plasmacytic	Plasmablast	Myeloblast, hemocytoblast, lymphoidocyte, lymphocyte, stem cell, lymphoblastic plasma cell, myeloma cell
	Proplasmacyte	Türk cell, Türk irritation form, lymphoblastic or myeloblastic plasma cell, myeloma cell
	Plasmacyte	Plasma cell, Unna's plasma cell, Marschalko's plasma cell, plasmacytoid lymphocyte, myeloma cell

TABLE 2—*Concluded*

NAME OF SERIES	TERM TO BE USED	TERMS TO BE AVOIDED
Thromboeytic	Megakaryoblast	Megalokaryoblast
	Promegakaryocyte	Premegalokaryocyte
	Megakaryocyte	Megalokaryocyte
	Thromboeyte	Platelet, thromboplastid
—	Disintegrated cell	Senile cell, smudge, basket cell, smear cell, degenerated cell

The definitions decided on are as follows:

Lymphoblast: Any cell of the lymphocytic series having fine chromatin structure in the nucleus. Cells of blast morphology associated with prolymphocytes should be tentatively classified as lymphoblasts.

Prolymphocyte: Any cell of the lymphocytic series intermediate in morphology between the lymphoblast and the lymphocyte. It will always have too coarse a chromatin structure to fit the criteria for a blast and too fine a chromatin structure or too large a cell diameter to be classed as a lymphocyte. Usually, but not always, prolymphocytes are larger than 15 microns in diameter, which is the upper limit for the lymphocyte.

Lymphocyte: Any cell of the lymphocytic series having the morphology of those commonly found in the blood of healthy adults.

Monoblast: Any cell of the monocytic series having fine chromatin structure. Usually nucleoli are visible. Cells of blast morphology found in association with promonocytes should be tentatively classed as monoblasts.

Promonocyte: Any cell intermediate in morphology between the monoblast and the monocyte. It is differentiated from the monoblast by having an irregularly shaped nucleus and somewhat coarser chromatin structure, and from the monocyte by the presence of one or more nucleoli.

Monocyte: Any cell of the monocytic series having the morphology of those commonly found in the blood of healthy adults. It is differentiated from the promonocyte by the absence of nucleoli.

Myeloblast: Any cell of the granulocytic series having fine chromatin structure and containing no specific granules. Usually nucleoli are visible. Cells of blast morphology found in association with progranulocytes should tentatively be classed as myeloblasts.

Progranulocyte: Any cell of the granulocytic series which has a nuclear structure too coarse for that of a blast cell and which has not yet developed discernible, specific granules. This term was selected rather than "promyelocyte" because of its clear relationship to the definition of granulocyte, given below, and because

the term "promyelocyte" has been in wide use for cells which do contain specific granules. The reason that the terms "granuloblast", "granulocyte", and "metagranulocyte" were not chosen was that the terms "myeloblast" and "myelocyte" were already in general use with essentially the definitions here given. This is true also for the term "granulocyte", which would otherwise have to be synonymous with the term "myelocyte".

Specific granules: Neutrophilic, eosinophilic or basophilic granules. This term does not include azurophilic granules.

Granulocyte: An inclusive term to apply to any cell containing specific granules. The plural form *granulocytes* would therefore include all myelocytes, metamyelocytes, band cells and segmented cells whether neutrophils, eosinophils, or basophils.

Myelocyte: Any cell containing specific granules, with a round or oval nucleus. It is distinguished from the progranulocyte by the presence of specific granules and from the metamyelocyte by the absence of indentation in the nucleus. It may be further subdivided, at the option of the user, into early and late stages, but the definition of early or late should be clearly stated in any publication.

This and all subsequent cells of the granulocytic series should be additionally characterized as neutrophil, eosinophil or basophil.

Metamyelocyte: Any cell of the granulocytic series having specific granules in the cytoplasm and a nucleus intermediate in shape between that of the myelocyte and the band cell. The nucleus usually has an indented oval shape, resembling a bean or kidney.

Band cell: Any cell of the granulocytic series which has a nucleus that could be described as a curved or coiled band, no matter how marked the indentation, if it does not completely segment the nucleus into lobes connected by a filament. It is differentiated from the metamyelocyte by an appreciable length of the nucleus having parallel sides, and from the segmented neutrophil by having no indentation which could be described as a filament.

Segmented cell: Any cell containing specific granules in which the lobes of the nucleus are connected by a filament. A *filament* is defined as a threadlike structure. Since at times, in viewing a three-dimensional object from one direction, it is impossible to be certain whether two parts of the nucleus are connected by a filament or band, it is suggested that such cells always be placed in the segmented category, since this is the more differentiated and more common cell.

The term *toxic neutrophils*, followed by a 1 to 4+ designation, is recommended for the grading of toxic granules, basophilia of the cytoplasm, vacuoles and condensation of nuclear chromatin in the neutrophils, since its meaning is clear, although it is recognized that it is not an adequately descriptive term. The grading should depend more on the degree of change than on the percentage of the cells involved and should be recorded in the report whenever the degree of change exceeds 2+.

Plasmablast: Any cell of the plasmacytic series having fine chromatin structure in the nucleus. Cells of blast morphology found in association with proplasma-

cytes are usually seen only in plasmacytic leukemia or multiple myeloma. The cytoplasm tends to be more opaque in staining than in the other leukocytic blast cells.

Proplasmacyte: Any cell of the plasmacytic series with a nuclear structure too coarse for that of a blast cell but with one or more nucleoli present.

Plasmacyte: A cell characterized by extremely coarse chromatin structure, with the deeply staining chromatin of the nucleus aggregated into large, sharply demarcated clumps. It is differentiated from the proplasmacyte by the absence of nucleoli. The cytoplasm of all cells of the plasmacytic series tends to be deeply basophilic and opaque in appearance. Azurophilic granules may be present or absent, but are more commonly absent.

Megakaryoblast: Any cell of the thrombocytic series having a nucleus with fine chromatin structure. Usually these are larger than the other blast cells.

Promegakaryocyte: Any cell of the thrombocytic series with a nucleus containing nucleoli but having a chromatin structure too coarse for a blast cell. The nucleus is usually similar in shape to that of the megakaryocyte. Fine azurophilic granules are usually diffusely scattered through the cytoplasm.

Megakaryocyte: Any nucleated cell of the thrombocytic series in which nucleoli are not discernible. The azurophilic granules are often aggregated into clumps. Megakaryocytes and promegakaryocytes are typically much larger than other cells found in the marrow.

Thrombocyte: Any cell of the thrombocytic series containing no nucleus; in other words, any non-nucleated fragment of megakaryocytic cytoplasm containing azurophilic granules similar to those of the mature megakaryocyte.

The term *thromboplastid* was recognized as being anatomically correct, but it was felt that to be consistent with the use of the term *erythrocyte* and to permit the use of "thrombocytic" and "erythrocytic" in describing these cell series, the suffix "cyte" was preferable for these two non-nucleated forms.

Disintegrated cell: Any cell of any series in which the cytoplasmic outline has been disrupted or the nuclear chromatin is no longer surrounded by a membrane, excluding the changes in the nucleus that occur in mitotic division. Disintegrated cells should be recorded as such in the differential report, even though they could be identified by dispersed granules. They should be counted even if only shreds of nuclear material are discernible, since they are undoubtedly included in the total leukocyte count.

It is, of course, understood that modifying adjectives may be applied to any of the recommended terms in describing results of investigation, but if these terms are to gain general acceptance they should not be given any new definitions except by general action of the committee.

All readers of this article are urged to express their approval, disapproval or suggestions for modification of the recommendations here published, by writing to Dr. A. S. Giordano, Secretary, American Society of Clinical Pathologists, 531 North Main Street, South Bend 1, Indiana, and sending a carbon copy of the letter to the chairman of the committee, Dr. Edwin E. Osgood, University of Oregon Medical School, Portland 1, Oregon.

An announcement of the response to this report will appear in a subsequent issue of this journal. Any further recommendations of the committee will be published in a similar manner.

At the next meeting of the committee, the nomenclature of diseases of the blood and blood-forming organs will be discussed. At subsequent meetings questions of terminology will be discussed for cells of the erythrocytic series and of the reticuloendothelial system and for other cells rarely encountered in blood or marrow but not infrequently seen in lymph node sections and imprints.

This report has been approved for publication by the following members of the committee:

Howard L. Alt	Russell L. Haden
Lawrence Berman	Byron E. Hall
O. A. Brines	F. J. Heck
C. G. Culbertson	J. M. Hill
T. J. Curphey	Roy R. Kracke
William Dameshek	L. R. Limarzi
Charles A. Doan	Stacy R. Mettier
Hal Downey	J. J. Moore
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R. F. Farquharson	Isabella H. Perry
Morris Fishbein	Richard J. Plunkett
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QUANTITATIVE ESTIMATION OF BARBITURATES IN BLOOD BY ULTRA-VIOLET SPECTROPHOTOMETRY.

I. ANALYTICAL METHOD*

J. T. WALKER, PH.D., R. S. FISHER, M.D., AND J. J. McHUGH, B.S.

*From the Chemical Laboratory of the Massachusetts Department of Public Safety and the
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INTRODUCTION

Investigation of the toxicity and metabolic fate of the barbiturates has been greatly hindered by the absence of a satisfactory analytical method. With the advent of the short acting barbiturates which appear in urine only in very low concentrations, the clinical problem of establishing the diagnosis of barbiturate poisoning in the absence of adequate history has become increasingly difficult. In medico-legal cases of unexplained deaths, the problem is particularly vexing since the postmortem examination in barbiturate fatalities rarely discloses characteristic anatomic lesions; and the chemical identification and quantitation of barbiturates in the blood or organs of the deceased may be the unique criterion in explaining the death.

Perhaps the most widely used clinical method of determination has been the Koppanyi adaptation of the cobalt color reaction.⁷ This group of reactions depends on the formation of a color when a chloroform extract containing the barbiturate is treated with methyl alcoholic solutions of cobaltous acetate and a base such as barium hydroxide or lithium hydroxide. However, it has been shown by Riley and co-workers⁹ that these reactions are nonspecific for barbiturates and that other substances frequently present in biologic materials may lead to false positive reactions. The classic Stas-Otto procedure, commonly used in medico-legal toxicology for the recovery of barbiturates,¹⁰ requires the extraction of large volumes of blood or organs in order that a crystalline barbiturate may eventually be recovered, characterized and weighed. This procedure was fairly satisfactory, although time-consuming, when barbital and phenobarbital were the only members of the series encountered. Many of the newer compounds, however, have much lower melting points or are deliquescent; or, because of their greater physiologic or toxic potency, occur in such low concentrations that they are crystallized from the substrate only with great difficulty. Another defect of this technic is the limitation of accuracy in quantitation imposed by the necessity of wasteful purification; the purer the product weighed, the less the recovery. To attain reasonable precision, it is necessary to use quantities of blood, tissue or urine of the order of 100 to 1000 Gm.³ Such amounts are not generally available, except at autopsy.

It has been recognized for a number of years that barbital and certain other 5,5-dialkyl substituted barbiturates, when dissolved in strongly alkaline aqueous

* Received for publication, January 19, 1948.

solution, possess a characteristic absorption band in the ultra-violet region of the spectrum.¹ This fact has been employed by Elvidge² as the basis of a spectrophotometric method of assay of barbiturate tablets.

The early authors, and recently Klotz and Askounis,⁶ variously describe the band maximum as occurring between 245 $m\mu$ and 255 $m\mu$ when the barbiturate is dissolved in alkali of 0.1 to 1.0 normality. The occurrence of an absorption band in alkaline solutions, and not in acid solutions, is ascribed by Klotz and Askounis to resonance of the molecule permitted by ionization of one of the nitrogen-bound hydrogen atoms. Since there are two equivalent ionizable nitrogen-bound hydrogen atoms in the barbiturate ring, di-enolization is also a formal possibility. The effect of varying alkalinity on the location and intensity of the ultra-violet absorption band and the possible formation of a di-enolic resonant state have not been reported.

Recently, Hellman and co-workers⁴ described an ultra-violet spectrophotometric technic for the estimation of pentothal in blood. Their procedure makes use of a characteristic absorption band at 2880 Å (288 $m\mu$) in ether solution. Their technic, however, is applicable only to the thiobarbiturates, and they state that "no other barbiturate tested gave any significant absorption in concentrations tested". Jailer and Goldbaum⁵ have subsequently modified the procedure of Hellman and co-workers and suggest that the measurement of the ultra-violet absorption of pentothal be carried out in chloroform or in a dilute aqueous alkaline solution. When the measurement is made in 0.2 N sodium hydroxide solution, the maximum absorption occurs at 305 $m\mu$.

It is the purpose of this paper to describe the effects of varying hydrogen ion concentration on the absorption of ultra-violet light by the barbiturates, to relate these effects to corresponding ionic states and to describe an analytical procedure for the determination of barbiturates in blood based on these phenomena.

THE NATURE OF THE ULTRA-VIOLET ABSORPTION BAND OF BARBITURATES IN ACID AND ALKALINE SOLUTIONS

Figure 1 shows the ultraviolet absorption curves for barbital at various hydrogen ion concentrations from pH 2 to pH 12. In strongly acid solutions the curves show no tendency to absorb ultra-violet light at 239 $m\mu$. For the purposes of this discussion, the molecular structure corresponding to the absorption curve in strongly acid solutions will be called the acid form. This probably represents the barbiturate in an un-ionized or lactam state. At pH 7 it is apparent that an equilibrium is present between the acid form and a second form exemplified by the curve at pH 10. This molecular state, which may be called the first alkaline form, probably represents the barbiturate in the first ionized or lactim state. The curves at pH 9 and pH 11 are essentially similar to that at pH 10. At pH 12 it is apparent that the barbiturate molecule has undergone a further change, probably due to a second stage of ionization. The absorption maximum shifts toward the visible region of the spectrum, the absorption band broadens at the base and the molecular extinction decreases. It was the second alkaline form

which gave rise to the curve reported by Klotz and Askounis.⁶ All of the 5,5 di-substituted barbiturates tested, including amytal, seconal, neonal, pentobarbital, phenobarbital and dial, give rise to curves similar to barbital over the pH range from 2 to 12 and, therefore, are capable of existing in the same 3 molecular states. As might be expected, evipal, a 1,5,5 tri-substituted barbiturate, is capable of existing only in the acid or un-ionized form and in the first alkaline form. Its absorption spectrum in alkaline solutions does not show a shift of

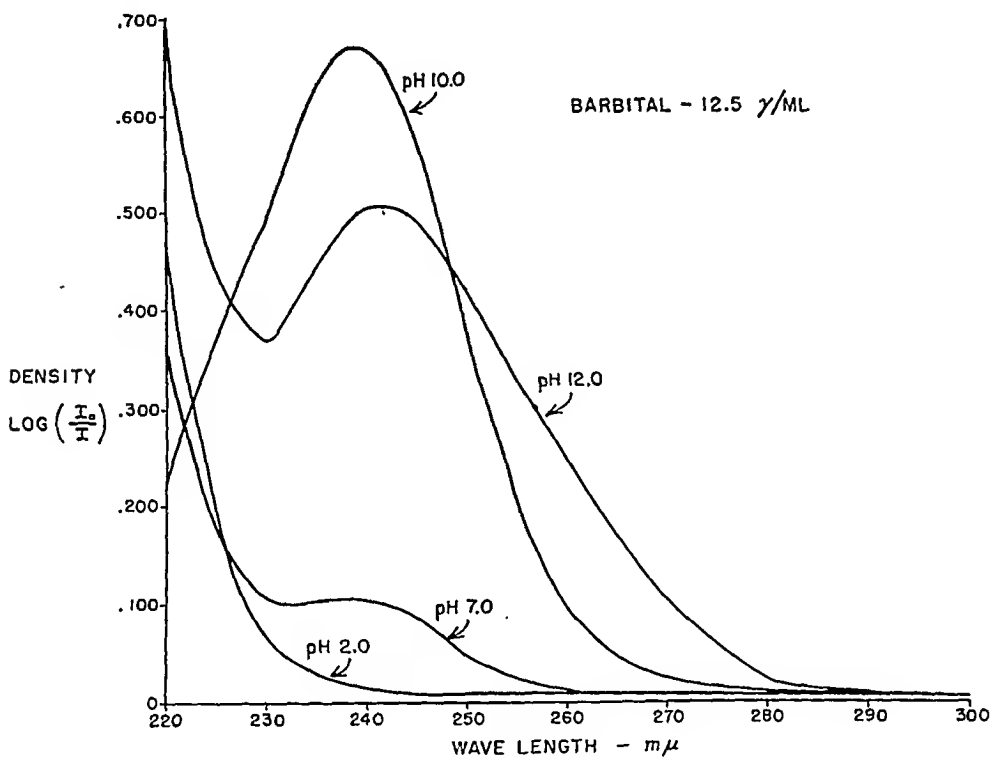


FIG. 1. ULTRAVIOLET ABSORPTION SPECTRA OF BARBITAL IN AQUEOUS SOLUTION OF VARIOUS DEGREES OF pH

the maximum from 239 $m\mu$ to 245 $m\mu$ when the pH is increased from 10 to 12. Further studies of the relationship of the structure of various barbiturates to their absorption of ultra-violet light are in progress.

That the magnitude of the absorption maximum of pentobarbital at 239 $m\mu$ in aqueous solution at pH 10 is proportional to the concentration, is shown by Figure 2 where maximal density is plotted against concentration over the range of 2.5 to 25.0 gamma per ml. Other barbiturates studied also conform to the Lambert-Beer equation. The calculation of the concentration of a pure aqueous solution of a given barbiturate from observed density readings at 239 $m\mu$ is, therefore, justified.

Table 1 shows the optical density (observed readings of $\log \frac{I_0}{I}$ at 239 $m\mu$) of a series of different barbiturates each present in concentration of 12.5 γ /ml. and

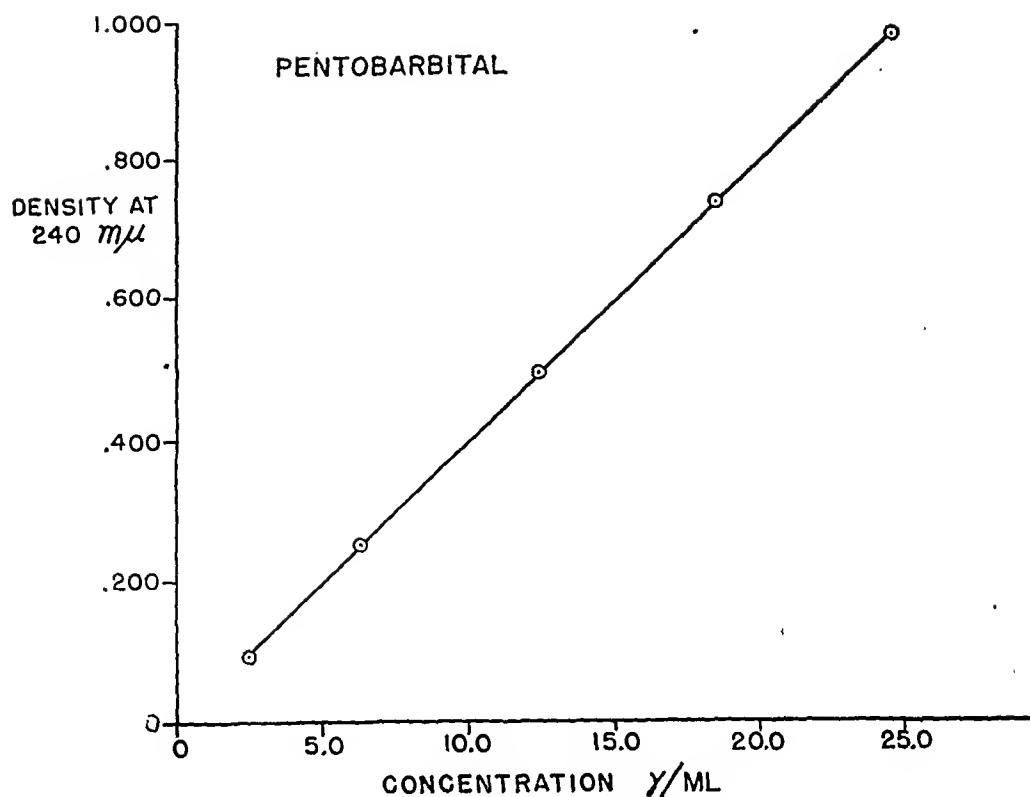


FIG. 2. RELATION OF OPTICAL DENSITY TO CONCENTRATION OF PENTOBARBITAL IN ALKALINE SOLUTION

TABLE 1
MOLECULAR EXTINCTION OF VARIOUS BARBITURATES (AND OBSERVED DATA)

SUBSTANCE	MOLECULAR WT.	MILLIMOLES LITER*	OBS. MAX. DENSITY (d_{alk}) pH 10.0-239 mμ	MOLECULAR EXTINCTION, ϵ_{max} .
	<i>M</i>			
Barbital	184.2	.0678	.692	10,200
Neonal.	212.2	.0588	.590	10,000
Pentobarbital.....	226.3	.0552	.552	10,000
Amytal.....	226.3	.0552	.557	10,000
Dial	208.2	.0600	.567	9,450
Seconal	224.3	.0557	.504	9,040
5-ethyl-5- β -methyl-allylbarbituric acid.....	210.2	.0594	.560	9,450
Phenobarbital	232.2	.0539	.584	10,800 (9,500)†

* All solutions were 12.5 γ /ml. of commercial products.

† Value in parentheses represents difference between ϵ_{max} in alkaline solution and molecular extinction in acid solution at same wave length (See page 455).

at the same pH (circa 10.0). The corresponding molar concentrations are shown, and the molecular extinctions have been calculated. If the identity of a barbiturate (in pure solution at pH 10.0) is known, its concentration (mg. per cent)

may be calculated from the molecular weight, the optical density of its solution and the molecular extinction shown (Equation 1, below).

If other chromogens in unknown amounts are present, the concentration of the barbiturate cannot be determined by simply measuring the optical density of the solution at pH 10 and at 239 $m\mu$. A correction must be made for the contribution to the density by the unknown component. In the case of blood extracts this correction may be readily accomplished by simply subtracting the optical density in acid solution (pH 2) from that in alkaline solution (pH 10); for it has been found that normal blood extracts, obtained by the method outlined below, exhibit almost identical optical densities in alkaline and acid solutions (Fig. 3).

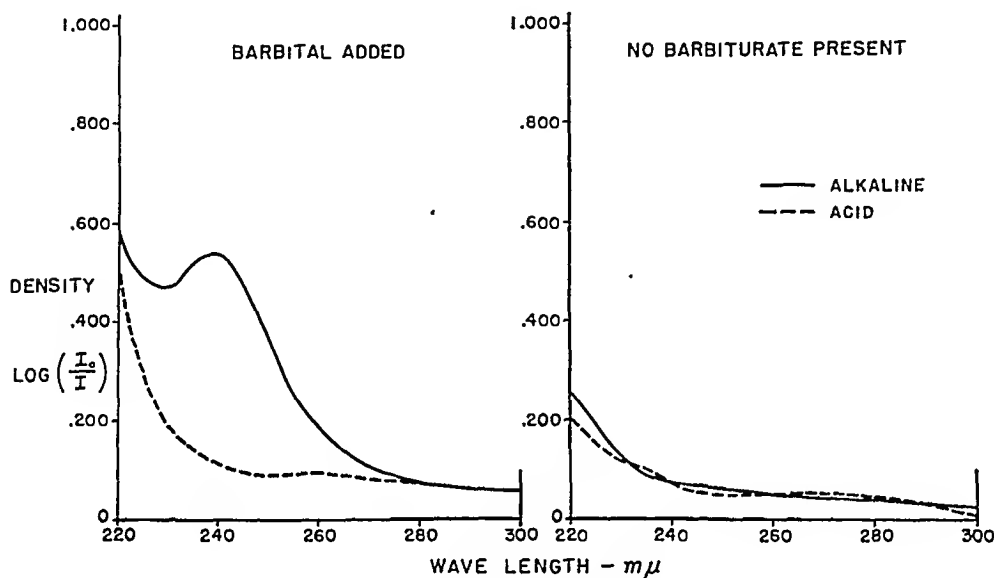


FIG. 3. ULTRAVIOLET ABSORPTION SPECTRA OF ALKALINE (pH 10) AND ACIDIFIED (pH 2) BLOOD EXTRACTS

As will be noted from Figure 1, the absorption of ultra-violet light by barbital at pH 2 is negligible in comparison to its absorption at pH 10. This relationship holds for all other barbiturates tested with the exception of phenobarbital. The latter compound showed an optical density in acid solution of approximately 12 per cent of that in alkaline solution. In calculations involving blood extracts where the differences between density readings in alkaline and acid solutions are used to quantitate the barbiturate concentration, a 13.6 per cent correction must, therefore, be made in the case of phenobarbital. This has been indicated in Table 1 by the value (9500) in the molecular extinction column.

The similarity in magnitude of the values of molecular extinction of each of the barbiturates (Table 1) validates the calculation of the molar concentration of an unknown barbiturate in blood from the difference between its alkaline and acid densities. (A molecular extinction of 10,000 may be assumed with less than 10 per cent error.) If the molecular weight of the unknown barbiturate is

assumed to be 225, the concentration by weight may be calculated within similar limits, except in the case of barbital, where, because of the low molecular weight, such an assumption would lead to an error of 24 per cent.

METHOD

A tungstate filtrate of the blood to be analyzed is prepared. The barbiturate is removed from an aliquot of the filtrate by successive chloroform extractions. The chloroform is dried with sodium sulphate and extracted once with dilute alkali. The pH of the alkali solution is adjusted to the range 9.0 to 10.5 by addition of sulfuric acid, and the absorption spectrum of the resulting solution is determined. It is then acidified and its absorption redetermined. The decrease in absorption at $239\text{ m}\mu$ on acidification is proportional to the barbiturate present in the original blood. As in other ultra-violet technics, maximum purity of all reagents is necessary.

Apparatus

A Beckman Model DU Ultra-violet Spectrophotometer with 1 cm. quartz cells was used for all optical density determinations. The wave length scale was calibrated against an alcoholic solution of benzene.

Reagents

10 per cent sodium tungstate CP $\frac{W}{V}$

10 per cent sulfuric acid CP $\frac{V}{\bar{V}}$

0.05 per cent sodium hydroxide

Chloroform, Reagent Grade, washed with acid and alkali and redistilled

Water, redistilled from glass

PROCEDURE

1. To 5.0 ml. of blood in a 50 ml. flask are added 30.5 ml. of water, 1.0 ml. of 10 per cent sodium hydroxide and 10 ml. of 10 per cent sodium tungstate. The mixture is shaken and allowed to stand five minutes to insure laking. Then, 3.5 ml. of 10 per cent sulfuric acid is added slowly with shaking. The flask is closed with a clean rubber stopper and shaken to insure homogeneity. The resulting solution is filtered through a dry filter paper (Whatman #4), the first portion of filtrate being returned to the original mixture in order to avoid cloud. Of the clear filtrate obtained, a 25 ml. portion is transferred to a clean 60 ml. separatory funnel. The use of stopcock lubricant must be avoided.

2. The aliquot is extracted serially by thorough shaking with three 10 ml. portions of purified chloroform. The chloroform is drawn off into a second separatory funnel through a funnel which has the stem stopped with a pledget of cotton on top of which a small amount of anhydrous sodium sulphate has been sprinkled.

3. The combined dried chloroform extracts are extracted once by thorough

shaking (three minutes) with 4.0 ml. of a 0.05 per cent sodium hydroxide solution. The chloroform is drained, and the supernatant alkaline solution containing the barbiturate is transferred to a test tube. A fine stream of nitrogen gas is bubbled through it for thirty seconds to rid the solution of chloroform. The pH is then adjusted to 9.0–10.5 by addition of a micro drop of dilute sulfuric acid. (The exact quantity must be determined experimentally for the alkali used.)

4. The solution is then transferred to a quartz cell of the ultra-violet spectrophotometer, and the optical density is read (against 0.05 percent NaOH solution to which has been added the quantity of H_2SO_4 required to adjust the pH of the unknown) at suitable wave lengths from 220 to 300 $m\mu$. The range of 235 to 242 $m\mu$ is critical; but, it is desirable to scan the entire range, since the extraction procedure will pick up certain other drugs which absorb ultra-violet light and may be identified in the same extracts (See discussion). The density at 239–240 $m\mu$ is designated d_{alk} .

5. After determining the "alkaline absorption" (d_{alk}), a micro drop of 10 per cent sulfuric acid solution is added to each cell, its contents mixed by inverting, and the absorption spectrum is again plotted, observing especially the value at that wave length where maximal optical density was found in the alkaline extract (239 to 240 $m\mu$). This value is designated d_{ac} .

6. To calculate the concentration of barbiturate in the solution subjected to spectrophotometry, one may employ the formula:

$$\text{Conc., } \gamma/\text{ml.} = \frac{(d_{alk} - d_{ac}) \times M \times 1000}{\epsilon_{max.}} \quad (1)$$

To calculate the concentration of barbiturate in blood, employing the procedure outlined above:

$$\text{Conc., mg./100 ml.} = \frac{(d_{alk} - d_{ac}) \times M \times 160}{\epsilon_{max.}} \quad (2)$$

Since by the extraction procedure outlined above, only about 70 per cent of the barbiturate present is recovered (Table 2), a correction factor may be incorporated in equation 2:

$$\text{Conc., mg./100 ml. (Corr.)} = \frac{(d_{alk} - d_{ac}) \times M \times 230}{\epsilon_{max.}} \quad (2a)$$

In the above:

M = Molecular weight,

$\epsilon_{max.}$ = Molecular extinction at the wave length of maximum density (Table 1),

d_{alk} = Optical density of solution at pH 9.0–10.5 at 239 $m\mu$,

d_{ac} = Optical density of solution at pH 2.0–5.0 at 239 $m\mu$.

EXPERIMENTAL RESULTS

Table 2 shows the recovery of varying amounts of pentobarbital added to blood. Table 3 shows the recovery of 100 γ amounts of various barbiturates

added to 5.0 ml. samples of a known negative blood. The results are shown as γ recovered as well as per cent recovery, and the factor of 70 per cent recovery used in equation 2a is derived from these experiments.

Figure 3 shows typical curves obtained from negative blood and blood to which a barbiturate has been added. It will be noted that the acid and alkaline curves of the barbiturate-containing blood coincide at wave lengths above 280 $m\mu$, and that in the case of the negative blood, the curves are almost superimposed throughout the entire region of the spectrum studied. Occasionally, the acid curve of a barbiturate containing blood will be higher (greater density)

TABLE 2
RECOVERY OF PENTOBARBITAL AT DIFFERENT CONCENTRATIONS IN BLOOD

γ ADDED PER 2.5 ML. BLOOD	γ RECOVERED	PER CENT RECOVERY
10	7.3	73.0
50	34.5	69.0
100	68.5	68.5
200	133.6	66.8
375	256.2	68.3
Average per cent recovery		69.1

TABLE 3
RECOVERY OF VARIOUS BARBITURATES ADDED TO BLOOD

γ ADDED PER 2.5 ML. BLOOD		γ RECOVERED	PER CENT RECOVERY
Seconal	50	34.0	68.0
Neonal	50	32.2	64.4
Phenobarbital	50	37.8	75.6
Barbital	50	33.6	67.2
Pentobarbital	50	34.5	69.0
Amytal	50	36.3	72.6
Average per cent recovery			69.5

in the range above 280 $m\mu$. The usual explanation of this is that the solution has become slightly turbid on acidification. In this case it is desirable to repeat the entire procedure, taking particular care to obtain an optically clear tungstate filtrate.

DISCUSSION

In evaluating analytical technics for the determination of barbiturates, it is convenient to consider separately the two phases, *viz.*: (1) extraction from the blood or tissue and (2) quantitation of the barbiturate in the final extract.

Extraction

Blood studies. Koppanyi *et al.*⁷ describe the use of sodium tungstate-sulfuric acid protein precipitation with subsequent chloroform extraction of the fil-

trate as one means of recovering barbiturates from blood. They report recoveries of 90.8 to 93.7 per cent of 10 milligram quantities of sodium barbital added to 20 ml. of dog's blood. Our recoveries, using an essentially similar method of extraction, averaged 70 per cent (Tables 1 and 2). The fact that they utilized concentrations in the range of 50 mg. per 100 ml., as compared with our 0.4 to 15 mg. of barbiturate per 100 ml. of blood, prevents direct comparison of percentage recovery. In our experiments a significant amount of the barbiturate apparently remains in the protein precipitate. However, within the concentration range studied this loss appears to be relatively constant and may be compensated for in the final calculation.

The Hellman procedure⁴ avoids tungstate protein precipitation and extracts the barbiturate directly from buffered plasma with serial portions of ether. The ether extracts are yellowish brown in color and are "cleared" by washing with 0.5 M sodium bicarbonate. After this step the absorption spectrum is determined directly in ether solution. Ether is not a suitable solvent where the alkaline and acid solutions are to be examined in succession. Further, appreciable quantities of barbiturates other than pentothal are lost when their ether solutions are washed with aqueous sodium bicarbonate. This is due to ionization and consequent increased water solubility of the barbiturate at pH 7 and above (Fig. 1). (The pH of 0.5 M sodium bicarbonate is about 8.) Finally, the recovery of barbiturates from unwashed ether extracts prepared by Hellman's procedure into 0.05 per cent NaOH solution yields opalescent solutions unsuitable for use in the ultra-violet spectrophotometer.

Jailer and Goldbaum extracted buffered plasma, blood and homogenized tissue directly with chloroform, and, after filtering to remove water droplets, quantitated the pentothal by ultra-violet spectrophotometry of the chloroform layer. Quantitation of the barbiturates whose ultra-violet absorption maximum is at 239 $m\mu$ cannot be satisfactorily achieved in chloroform solution. This is due to the pronounced absorption of ultra-violet radiation by chloroform itself at this wave length. They offer the alternate procedure of extracting the chloroform with 0.2 N NaOH and quantitating pentothal in the alkaline solution. They state that "in all experiments control plasma was used as a blank (which is small). . .". Our experiments with this latter extraction procedure, using 1 ml. of plasma, gave blanks which at 239 $m\mu$ had optical densities varying from .180 to .480. Further, these extracts after acidification showed different (usually lower) optical densities at 239 $m\mu$ than when alkaline. This, of course, would lead to significant errors in determination of the blank (d_{ac}). We, therefore, discarded the direct extraction procedure.

Tissues. A few experiments have been done applying the procedure described for blood directly to brain and liver, using finely macerated tissue instead of a blood specimen. The results obtained suggest that the technic may be directly applicable to tissues.

Quantitation

The ultra-violet absorption technic offers a sensitive, quantitative method for estimating barbiturates when other chromogens are not present. That this is

the case is obvious both from the theoretical considerations of the nature of ultra-violet absorption phenomena and from the experimental results shown in Figure 2. In blood extracts other chromogens besides barbiturates are present and contribute to any spectrophotometric reading. The finding that non-barbiturate extractives show essentially the same ultra-violet absorption in acid and basic solutions, coupled with the fact that barbiturates absorb ultra-violet light at $239\text{ m}\mu$ only in alkaline solution, permits the determination of the fraction of the total absorption due to barbiturate *per se*.

The cobalt color reaction, as described by Koppanyi *et al.*, is stated to be sensitive to barbital in concentrations of $10\text{ }\gamma/\text{ml.}$ in the chloroform solution to be tested. The limiting concentration of barbital to yield satisfactory spectrophotometric readings is about one-fourth of this figure. With the present extraction technic this is equivalent to 0.4 mg. barbiturate per 100 ml. of the original blood. The limiting factor in sensitivity of the spectrophotometric method is the ratio of the barbiturates present to the other blood chromogens concomitantly extracted. Further refinements of extraction technic with the object of reducing the extraction of non-barbiturate chromogens may allow barbiturate quantitation in the order of less than 0.1 mg. per 100 ml. This latter would probably represent blood levels unaccompanied by clinical manifestation of barbiturate effects.

Direct comparison of our ultra-violet spectrophotometric technic with those of Hellman and Jailer and their co-workers cannot be made, since the ultra-violet absorption spectrum of pentothal differs markedly from that of the other barbiturates. Thus, pentothal in 0.05 per cent sodium hydroxide solution has two maximums, one at $255\text{ m}\mu$ and a much larger one at $305\text{ m}\mu$. Another characteristic of pentothal is the occurrence of strong ultra-violet absorption in acid solutions which prevents determination of the barbiturate absorption by simple subtraction of the density in acid from that in alkaline solution.

Interfering substances. Certain substances occasionally present in the blood show significant absorption in the ultra-violet region and may appear in the alkaline extract obtained by the technic described in this paper. Salicylates and sulfonamides appear to be the most important of these. Salicylic acid, however, shows maximums at $230\text{ m}\mu$ and $296\text{ m}\mu$ in alkaline solution and at $235\text{ m}\mu$ and $302\text{ m}\mu$ in acid solutions and would readily be recognized by the absorption at $296\text{ m}\mu$ and $302\text{ m}\mu$. Thus, its interference would not lead to false high barbiturate levels, but would, if salicylate is present in the blood in concentrations over 2 mg. per 100 ml. , prevent the detection of less than about 2.0 mg. per cent of barbiturate. It is true, however, that the presence of salicylate would be recognized from applying the technic. It is highly probable that a simple and accurate technic for determining salicylates in the blood can be devised, making use of the ultra-violet spectrophotometer and the facts noted above.

Sulfadiazine in concentrations of approximately 5 mg. per 100 ml. in the original blood will obscure the absorption maximum due to barbiturates in concentrations of 2.5 mg. or less per 100 ml. Conversely, when the concentration

of barbiturate reaches four times the sulfadiazine level, that portion of the curve contributed by the latter is obscured.

Picrotoxin, benzedrine and caffeine, commonly used stimulants in the treatment of barbiturate poisoning, either do not appear in the final extract using this technic, or do not exhibit significant absorption in the ultra-violet region and, therefore, do not interfere with the technic.

SUMMARY

1. A new application of the ultra-violet absorption technic for the estimation of the barbiturate content of blood and tissue is described. Comparison of this method with previously used procedures shows that it has significant advantages in relation to accuracy, simplicity and rapidity.

2. The ultra-violet absorption spectra of several 5,5 di-substituted barbiturates in aqueous solutions of different pH are described, and the characteristic changes due to varying pH are discussed.

Acknowledgments. We wish to thank Ciba Pharmaceutical Products, Inc., Eli Lilly and Company, Winthrop Chemical Company, Inc., and Abbott Laboratories for supplying us with the barbiturates used in this investigation.

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QUANTITATIVE ESTIMATION OF BARBITURATES IN BLOOD BY ULTRA-VIOLET SPECTROPHOTOMETRY.

II. EXPERIMENTAL AND CLINICAL RESULTS*

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The literature contains several reports of experimental studies relating concentrations of barbiturate in blood and tissue to the dosages administered. There are, however, little data available with regard to concentrations obtained after clinical use of these drugs. Also, clinical studies of the relation of blood concentration of a barbiturate to the resulting degree of central nervous system depression are not available. Such information is needed both to control clinical administration of the drugs and to evaluate prognosis in cases of barbiturate overdosage. The need has been especially emphasized by the advent of a relatively simple method of determining blood concentrations of barbiturates. This paper contains the results of experimental and clinical studies on barbiturates using the analytical technic for the determination developed in these laboratories.⁴

OBSERVATIONS

I. Experiment in Animals

Mongrel hounds of similar weight were given anesthetizing doses of sodium pentobarbital and barbital by stomach tube. The blood concentrations of the drugs were determined at intervals, and concomitant observations of the degree of depression were made. The results are shown in Table 1. With pentobarbital in a dose of 36.4 mg./Kg. (40 mg./Kg. as the sodium salt) the maximum blood concentration of 2.2 mg./100 ml. was reached in two hours, following which there was a plateau maintained for several hours. By the sixth hour the blood barbiturate concentration was beginning to decrease. After twelve hours there was partial return of consciousness with agitation and ataxia, and the blood concentration had fallen to less than 50 per cent of its maximum level. Only a trace remained at twenty-four hours.

In contrast to these results are those when barbital, 200 mg./Kg., was given. Here the blood barbiturate concentration continued to rise until the fourth hour, after which it decreased gradually, but barbiturate was still present in considerable quantity twenty-four hours after administration. Of more significance is the fact that with comparable effects on the central nervous system, the blood concentrations of barbital were between 7 and 9 times as high as those of pentobarbital. This difference is indicative of the marked variation in physiologic potency among the various barbiturates. It suggests, too, that the relative "slowness of action" of barbital is in large part due to the purely mechanical or

* Received for publication, January 19, 1948.

physical factors whereby a longer absorption time is necessary to attain the blood concentrations that are required to produce a given effect with this drug. The longer duration of effect would seem to be partly of the same "mechanical" nature, although the ultimate mechanism of detoxication obviously is also of major importance in this connection.

TABLE 1

BLOOD CONCENTRATION OF PENTOBARBITAL AND BARBITAL IN DOGS IN RELATION TO DEPTH OF CENTRAL NERVOUS SYSTEM DEPRESSION

HOURS AFTER ADMINISTRATION	PENTOBARBITAL,* 36.4 MG./KG.		BARBITAL* 200 MG./KG.	
	Blood conc.	Depth of depression	Blood conc.	Depth of depression
	mg./100 ml.		mg./ 100ml.	
1	1.8	Deeply comatose. Corneal reflex absent. No response to painful stimuli.	13.8	Still erect but with marked ataxia.
2	2.2	Same as 1 hr.	15.3	Comatose. Corneal reflex active. No response to painful stimuli.
3	2.2	Same as 1 hr.	15.8	Same as 2 hr.
4	2.2	Same as 1 hr.	17.6	Same as 2 hr. except corneal reflex slightly less active.
6	1.8	Same as 1 hr., beginning to whine slightly.	16.2	Corneal reflex active. Whined and stirred at venipuncture.
12	0.9	Eyes open. Thrashing about, unable to stand, resists venipuncture.	15.0	Eyes open. Thrashing about but unable to stand.
24	Trace <.15	Up and about. Normal.	11.8	Up and about but with marked ataxia.

* Given by stomach tube with 50 ml. of water per Kg. body weight after a twenty-four hr. fast. Water intake was not restricted.

II. Clinical Experiments in Human Subjects

A series of patients, undergoing the "amytal test" for evaluation of blood pressure response to sedation, was studied to determine the blood concentrations of amytal obtained. A second group of patients, who were receiving a new barbiturate (5-ethyl-5- β -methylallylbarbituric acid), under investigation as a therapeutic agent in essential hypertension, was similarly studied. The results are shown in Table 2.

Examination of the amytal results (Cases 1 to 6) shows that even in this small group of patients there was wide variation in the blood concentrations of amytal obtained in different patients under clinical conditions which were as nearly uniform as possible. This variation does not appear to be related to sex or weight. Patients 1 and 2 were of the same sex, weight and age, and received identical amounts of the barbiturate; they showed over 100 per cent difference in blood amytal concentration (0.40 mg. per 100 ml. versus 0.91 mg. per 100 ml.).

The clinical observation that certain patients undergoing this test cannot be readily aroused for some hours, whereas others remain conscious or sleep only lightly, would seem to correspond with the observed variations in blood barbiturate levels. The second group of patients (7 to 11), who ingested 5-ethyl-5- β -methylallylbarbituric acid, also showed a striking variation (from 0.26 to 1.00 mg. per cent) in blood concentrations after identical doses of the drug. Another feature worthy of comment is the fact that the patients of the second group

TABLE 2
BLOOD CONCENTRATION AFTER THERAPEUTIC DOSES OF SODIUM AMYTAL AND
5-ETHYL-5- β -METHYLALLYLBARBITURIC ACID

AGE	SEX	WEIGHT	HOURS AFTER ADMINISTRATION								REFERENCE NUMBER
			0	1	2			3			
			Dose	Dose	Accumulated total dose	Blood conc.	Dose	Accumulated total dose	Blood conc.		
Sodium Amytal*											
		Kg.	mg./Kg.	mg./Kg.	mg./Kg.	mg./100 ml.	mg./Kg.	mg./Kg.	mg./100 ml.		
53	F	63.1	3.2	3.2	6.4	0.25	3.2	9.6	0.40	1—M. S.	
53	F	61.9	3.2	3.2	6.4	0.66	3.2	9.6	0.91	2—R. S.	
53	F	70.6	2.8	2.8	5.6	0.38	2.8	8.4	0.64	3—D. D.	
47	M	79.5	2.5	2.5	5.0	0.33	2.5	7.5	0.53	4—S. L.	
51	M	71.8	2.7	2.7	5.4	0.28	2.7	7.1	0.33	5—J. M.	
48	M	85.8	2.3	2.3	4.6	0.63	2.3	6.9	0.67	6—J. E.	
5-Ethyl-5-β-Methylallylbarbituric Acid†											
27	F	51.2	1.9	1.9	3.8	—	1.9	5.7	0.43	7—E. A.	
55	M	76.4	1.3	1.3	2.6	0.70	1.3	3.9	0.75	8—S. F.	
54	M	72.2	1.3	1.3	2.6	0.70	1.3	3.9	1.00	9—S. B.	
48	M	76.4	1.3	1.3	2.6	0.51	1.3	3.9	0.73	10—H. T.	
31	M	73.2	1.3	1.3	2.6	0.29	1.3	3.9	0.26	11—C. G.	

* Given 3 grain capsules of sodium amytal successively at "0" hr., "1" hr. and "2" hr. Blood samples were drawn at time of third dose and one hour thereafter.

† Given 5-ethyl-5- β -methylallylbarbituric acid on same schedule, but each dose consisted of 1.5 grains of the drug.

received only one-half as much barbiturate as those in the sodium amytal group, but they exhibited higher blood barbiturate concentrations (average of three hour amytal levels 0.50 mg. per cent; average 5-ethyl-5- β -methylallylbarbituric acid 0.63 mg. per cent).

III. Non-fatal Poisoning in Human Subjects

Several patients, who had ingested large doses of barbiturates with presumably suicidal intent, were studied from the time of admission until they had recovered consciousness. They are grouped in Table 3 according to the identity and ap-

TABLE 3

BLOOD CONCENTRATION AFTER SUICIDAL (OR ACCIDENTAL) INGESTION OF NON-FATAL
DOSES OF VARIOUS BARBITURATES

NAME	SEX	AGE	DRUG	AMOUNT .	HRS. AFTER INGESTION (APPROX.)	BLOOD BARB. CONCEN. .	CLINICAL STATE OF INTOXICATION*
		yr.		grams		mg./100 ml.	
L. W.	F	41	Seconal	1.9	36	0.5	Awake
N. P.	F	60	Seconal	0.6	6	0.8	Awake
					12	0.8	Awake
R. O.	F	28	Seconal	1.0	12½	1.8	Comatose
			(plus several amytal capsules and a few phenobarbital tablets)		18	1.5	Awake
W. H.	F	34	Pentobarbital	2.0	16	1.6	Comatose
					22	1.0	Comatose
					28	0.95	Awake
					38½	<0.5	Awake
R. H.	M	21	Pentobarbital	0.8	4	1.4	Comatose
					10	0.8	Awake
Z. B.	F	23	Pentobarbital	?	2	1.4	Comatose
					16	1.6	Comatose
					48	—	Awake
W. R.	M	43	Phenobarbital	?	16	12.8	Comatose
					22	11.8	Awake
					34	11.2	Awake
L. R.	F	60	Phenobarbital	3.2	60	10.4	Comatose
D. I.	F	26	Barbital	?	66	5.8	Awake
R. D.	M	40	Barbital	?	31	20.6	Deeply comatose
			(Quantity not known. Suicidal intent denied. Identity of drug recovered from urine established as barbital by mixed melting point with known sample of barbital.)		41	19.1	Deeply comatose
					53	16.4	Deeply comatose
					59	15.4	Deeply comatose
					65	13.2	Deeply comatose
					77	11.8	Comatose
					83	10.8	Comatose
					89	8.9	Comatose
					101	8.5	Comatose
					107	7.6	Awake
					113	7.1	Awake
					125	6.3	Awake
					131	6.3	Awake
					137	6.3	Awake
					149	5.4	Awake
					173	2.6	Awake
					197	1.8	Awake
					221	1.7	Recovered

* Graded at three levels, viz.: Deeply comatose, indicates total areflexia including loss of respiratory and pupillary response on strong supraorbital pressure; Comatose, indicates a state in which tendon and/or cutaneous reflexes were present but there was no response to the spoken voice and no oral response to painful stimuli; Awake, indicates oral response to spoken voice and includes several instances in which the patient was extremely drowsy but could be awakened or was awake but not clearly oriented. No patients completely free of barbiturate effects were included in this category.

proximate dosage of the barbiturate. Blood concentrations and concomitant observations of state of cerebral depression are indicated.

The first in the second series (L. W.) had taken a dose sufficient to cause coma of twenty-four hours' duration and had awakened prior to collection of the blood specimen reported. Nevertheless, she was still confused and had slurring of speech. The second patient (N. P.) was treated with stimulant drugs and, although quite drowsy at six hours, she did not become disoriented. The blood level attained and the clinical state of the patient were similar in general to those encountered in the patients undergoing the "amytal sedation tests" (Table 2). In the third patient (R. O.) the presence of an undetermined amount of phenobarbital in the mixture ingested probably led to a higher barbiturate level at the time of awakening than would be obtained had the drug been secondal alone.

In the pentobarbital cases the lowest observed blood barbiturate levels accompanied by coma were 1.0, 1.4 and 1.4 mg. per 100 ml., and those subsequent to or at the time of awakening were in the neighborhood of 1.0 mg. per 100 ml. Inasmuch as no significant quantity of barbiturate was recovered in gastric lavage of the second patient in this series (R. H.) and the first blood specimen was obtained after four hours, it is probable that the concentration of 1.4 mg. per 100 ml. is maximal for this dosage. Likewise our experience with this and other short-acting barbiturates would lead us to believe that in patient W. H. the maximal blood concentration had been passed prior to collection of the first blood specimen at sixteen hours.

The phenobarbital and barbital patients may be considered together, and they show quite a different magnitude of blood barbiturate concentrations than those with the short-acting barbiturates. With the long-acting barbiturates the return of consciousness at blood levels of 11.8 mg. per 100 ml. (W. R.) and 7.6 mg. per 100 ml. (R. D.) again illustrates their lesser physiologic or toxic potency as compared with their more rapidly acting relatives. The second phenobarbital patient, L. R., although comatose at the time of the blood barbiturate determination, was not deeply depressed and subsequently recovered. The fact that L. R. was comatose with 10.4 mg. of phenobarbital per 100 ml. of blood, whereas W. R. awakened with a blood concentration of 11.8 mg. of the same drug, may be due to normal physiologic variation or to other factors. These include the age and sex differences as well as the fact that L. R. had not used phenobarbital frequently prior to this hospitalization, whereas W. R. had been taking the drug in moderate dosage for a prolonged period.

In the case of R. D. it was possible to obtain much of the urine excreted during the first six days of his hospitalization. These specimens were analyzed for barbiturate, and the composite results appear in Figure 1. The graph of blood barbital levels is a relatively smooth curve with the decrease in blood barbital in the first two and one-half days of observation, averaging 0.2 mg. per 100 ml. per hour, while in the second half of the observation period, the rate approached 0.05 mg. per 100 ml. per hour. The urine barbital concentration varied from 300 in the first specimen to 12.1 mg. per 100 ml. in the last. The total amount of barbital recovered in the five day period amounted to 7.4 grams! In spite

of the extremely high blood levels encountered and the large amount of barbital ingested, the patient made an apparent clinical recovery and was discharged from the hospital without gross evidence of residual cerebral damage.

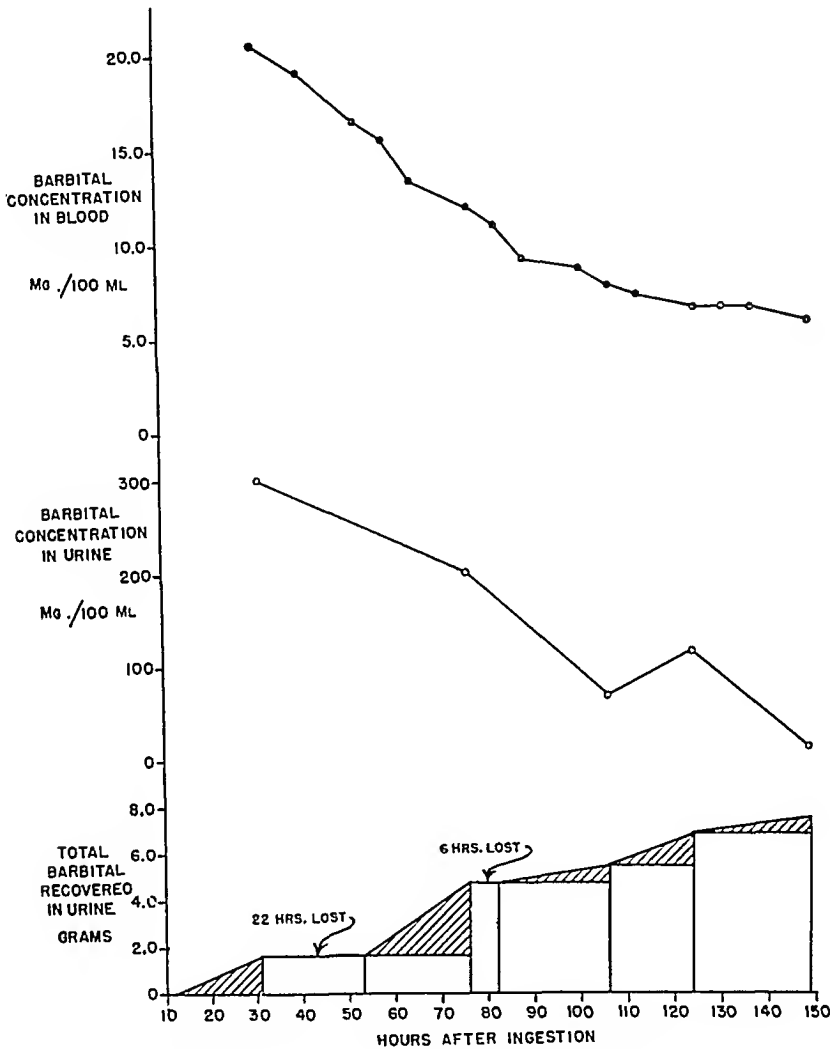


FIG. 1. BLOOD AND URINE BARBITAL CONCENTRATIONS AND URINARY RECOVERY OF BARBITAL IN A CASE OF BARBITAL POISONING

The shaded areas in the lower curve represent increments of barbiturate in the periods indicated.

IV. Human Subjects: Fatal Poisoning

In the routine work of these departments, deaths caused or contributed to by barbiturates are frequently encountered. Table 4 presents a series of such cases with the barbiturate concentrations determined in postmortem blood or tissue. None of the subjects in this group showed evidence of significant disease on complete postmortem examination.

The cases fall into 2 general groups based on blood barbiturate concentrations.

The first 4 subjects with barbiturate levels of 2.5 mg. or less per 100 ml. all showed concomitant alcoholism, and neither the barbiturate nor the alcohol concentration would ordinarily be considered as fatal levels. In the third case (7820), the lapse of time between ingestion of noxious agents and death was known accurately to be only one and one-half hours. The rapid onset of coma with prompt death in this case and the fatal outcome of the other cases, despite the absence of ordinarily fatal concentrations of either alcohol or barbiturate, is impressive and

TABLE 4

BLOOD CONCENTRATIONS AFTER SUICIDAL OR ACCIDENTAL INGESTION OF FATAL DOSES OF VARIOUS BARBITURATES

CASE	HISTORY	BARBITURATE LEVEL DETERMINED
		mg./100 ml.
8759	Adult. Found dead. Chronic alcoholic. Also taking pentobarbital. Blood alcohol 0.17 per cent.	0.6
8633	Adult. Found dead. Alcoholic and habitual user of pentobarbital. Blood alcohol 0.37 per cent.	0.7
7820	35 year old male. Ingested 0.8 Gm. of neonal and some whiskey. Died one and one-half hr. later. Blood alcohol 0.13 per cent.	1.0-2.0*
9661	Adult female. Found dead in bed with bottle of pentobarbital capsules at bedside. Blood alcohol 0.22 per cent.	2.5
8905	Adult male. Admitted to hospital in coma. Died without regaining consciousness forty-eight hr. later.	4.8
9485	Female 29 years of age. Found dead in woods. Missing eight days. All organs showed marked decomposition.	6.6
8691	Adult female. Barbiturate suicide.	7.2
9658	Barbiturate suicide. Found dead in hotel bathroom. Stomach contained several grams of barbiturate.	12.0
8926	Barbiturate suicide. Died two days after ingestion of unknown quantity of phenobarbital and pentobarbital. Analysis of brain tissue.	12.3
7732	Ingested large amount of barbiturate in bathroom. Walked to living room, collapsed and died in thirty minutes. Barbiturate identified as pentobarbital.	58.0*

* Indicates barbiturate determinations by Stas Otto-gravimetric procedure prior to development of present technique.

should focus attention on the very real danger of prescribing heavy barbiturate sedation for alcoholics who are trying to "taper off". This phenomenon has been commented on elsewhere in connection with experimental studies,³ and three deaths thought to be due to the "combined effects" of barbiturate and alcohol were reported from these laboratories in 1943.² We believe that a search for barbiturates, using the new techniques that detect small amounts of the drugs in "alcoholic" deaths, will reveal that the "combined effects of barbiturate and alcohol" are frequently the true cause of death. The implications in the clinical treatment of alcoholism are obvious.

The remaining 6 cases are in general similar to those of Gettler¹ and illustrate fatal blood concentrations of barbiturates. With the exception of Case #9485 all are known to have succumbed within three days of ingestion of the drug. Undoubtedly, in some individuals who survive longer, lower postmortem barbiturate levels will be encountered. We have observed fatality where second poisoning was the only clinically evident cause of death, with the blood second concentration being less than 1.0 mg. per 100 ml. on the fourth day of coma. Death ensued on the sixth day, but inasmuch as a postmortem examination was not obtained, the case is not included in Table 4.

SUMMARY

1. The concentration of barbiturate in the blood (determined by a new ultra-violet spectrophotometric technic) has been investigated with regard to the kind and dose of the drug and to the lapse of time after ingestion in a series of non-fatal and fatal cases (therapeutic, accidental, suicidal).

2. Coma may occur with blood concentrations as low as 1.0 mg. per 100 ml. after ingestion of certain short-acting barbiturates, whereas consciousness may be regained after ingestion of a slow-acting barbiturate with a residual blood concentration as high as 11 mg. per 100 ml.

3. Recovery from barbiturate poisoning (barbital) was observed in one instance in which more than 7.4 Gm. of the drug was excreted in the urine during the course of six days.

4. Examples are presented in which death was apparently due to the combined effects of alcohol and barbiturate, each of which was present in concentrations not ordinarily considered fatal. The danger incident to indiscriminate use of the two agents at the same time is stressed.

Acknowledgments. We wish to acknowledge our indebtedness to the various members of the medical and surgical staffs of the Massachusetts Memorial, Boston City, Boston Psychopathic, New England Deaconess and Beth Israel Hospitals and of the Medical Examiner's Office, First District, Middlesex County, Massachusetts, who obtained specimens the analyses of which are included in this report.

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THE EFFECT OF INSULIN ON THE BLOOD PICTURE*

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During the examination of a patient who was receiving insulin shock therapy for schizophrenia at the Colorado Psychopathic Hospital, a leukocytosis of 27,000 was found, and the question arose as to the possible relationship of this leukocytosis to the administration of insulin. Inquiries at several local institutions using this therapy failed to reveal an adequate answer to the problem. A search of the literature revealed several reports in foreign journals of the occurrence of a leukocytosis during shock produced by insulin.

Although all the reports mentioned an increase in the total white cell count during insulin therapy for mental aberrations, there was considerable disagreement concerning the differential picture. For example, the only American investigators, Levine and Kolars,⁵ using rabbits for experimental subjects, found no change in the differential count. Kugelman,⁴ Georgi² and Török⁷ reported a leukopenia preceding the leukocytosis during which time the differential count was unaltered. Boden and Wankell,¹ Sagel⁶ and Traczyński^{8,9} demonstrated a neutrophilia and a lymphopenia during the leukocytosis. During insulin therapy, Heilbrunn³ found an increasing neutrophilic leukocytosis, and a shift to the left, up to the time of interruption of the coma.

Because of the discrepancies reported in the differential picture and the paucity of English reports, it was deemed advisable to study the effect on the blood picture of the injection of insulin as used in the therapy of mental disorders.

METHODS

Studies for this paper were made on psychopathic patients at two local institutions, the Colorado Psychopathic Hospital and Psychiatric Department of the Fitzsimons General Hospital.

The investigations in Series I were confined to the leukocytic picture only, and were made on 20 schizophrenic patients undergoing insulin shock therapy at the Colorado Psychopathic Hospital. For such therapy the standard procedure at this hospital consists of daily injections of regular insulin to fasting patients at 7:00 a.m. The initial dose is usually 15 or 20 units, and on each succeeding day the dose is increased by 10 or 15 units until the desired state of coma is attained. About four hours after the injection of insulin, an adequate amount of sucrose is administered by means of gavage to terminate the coma. The patients bathe, eat their noon meal and spend the afternoon in routine activities. The 20 patients included in this series were chosen without regard to sex, the amount of insulin administered, or the number of days of previous treatment. Total white

* Presented at the Twenty-Sixth Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 29, 1947. Received for publication, October 29, 1947.

blood cell and differential counts were made on each of these patients one hour before and again immediately preceding the injection of insulin and the average of these determinations was taken as the individual's normal leukocyte value. Following the injection of insulin, total and differential counts were made at hourly intervals for ten hours. For each total white cell count two separate dilutions were made, using pipets previously standardized against government certified pipets. Counting chambers certified by the United States Bureau of

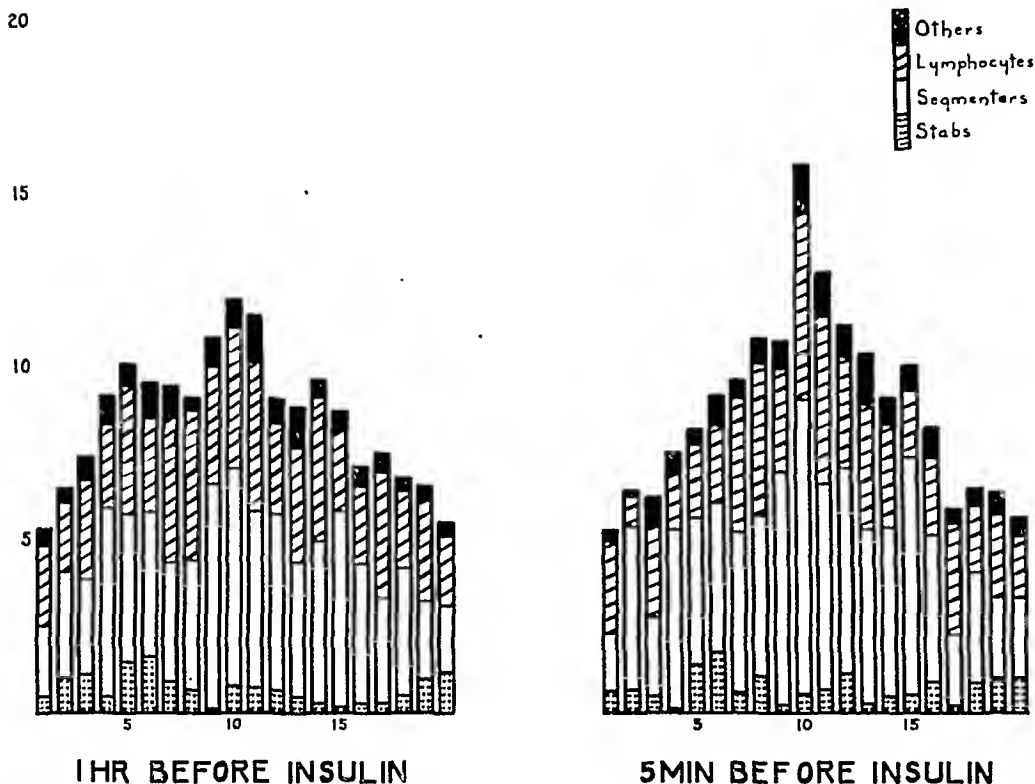


FIG. 1A. Figs. 1 A-D represent hourly leukocytic counts on 20 psychiatric patients undergoing insulin shock therapy, Series I. Individual cases are identically placed in each graph. Column height represents total white cell count; values of all components of the leukocytes are graphically shown. Numbers indicate thousands.

Standards were used for the counts, and each dilution was checked to within the normal range of error (500 cells). The thin blood films for the differential counts were made on glass slides by means of a special cover slip spreader and were stained by Wright's method. A Schilling count of 300 cells was made on each film.*

The investigations reported in Series II were made on 44 patients receiving insulin therapy in the Psychiatric Department of the Fitzsimons General Hospital. Of this group, 22 patients were receiving deep shock therapy (Group A) which entailed the administration of sufficient amounts of regular insulin to

* All counts in both series were made by the authors.

produce coma; the dose in this group of patients averaged 138 ± 49 units. The remaining 22 were receiving subshock therapy (Group B) in which insulin was given daily but in amounts insufficient to produce coma, and which never exceeded 90 units per day. In this group, the average dose was 52 ± 21 units. The Colorado Psychopathic Hospital studies (Series I) had established the occurrence of an insulin leukocytosis that reached its maximum sometime between the third and fifth hour and did not disappear within the period of study which extended ten hours after the injection of insulin. The sampling times for the

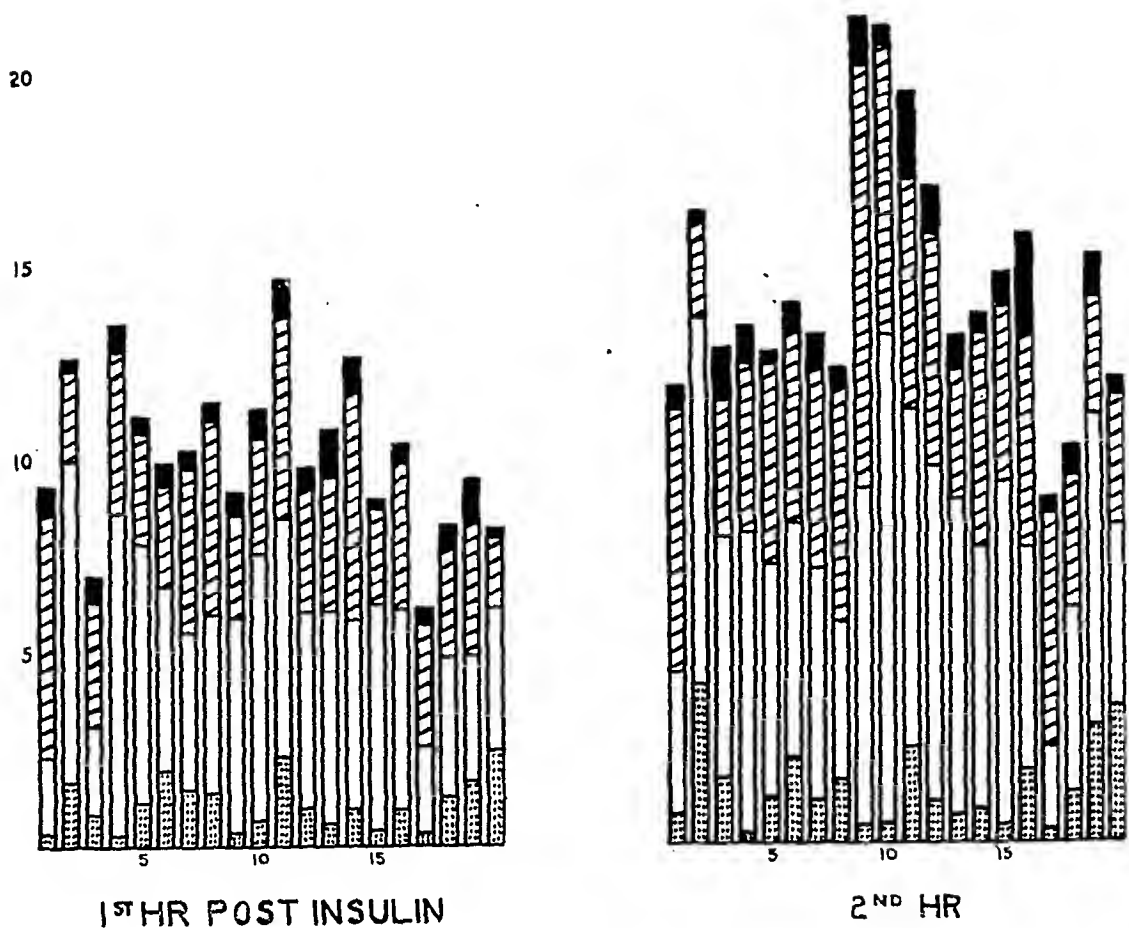


FIG. 1B

leukocytic studies on the patients in the Fitzsimons General Hospital (Series II) were subject to the following conditions. White blood cell and differential counts were taken on each patient one hour before insulin was administered to establish his normal values; four hours after insulin, at which time the maximum alteration in the leukocytic picture was anticipated; and again fifteen hours after injection of insulin to see if counts taken after this interval of time would show a return to normal. The same technics and precautions to assure correct counts were used in these total and differential determinations as were employed in Series I.

Since one cellular constituent of the blood, the leukocyte, was numerically altered by insulin, the possibility existed that other cellular elements also might be affected. Therefore, the effect of insulin on the red blood cell constituents

was studied on these patients by means of red blood cell counts, hemoglobin and hematocrit determinations and sedimentation rates. These determinations were carried out at the same time the leukocytic counts were made, except that at the fifteen hour postinsulin period no erythrocytic studies were conducted.

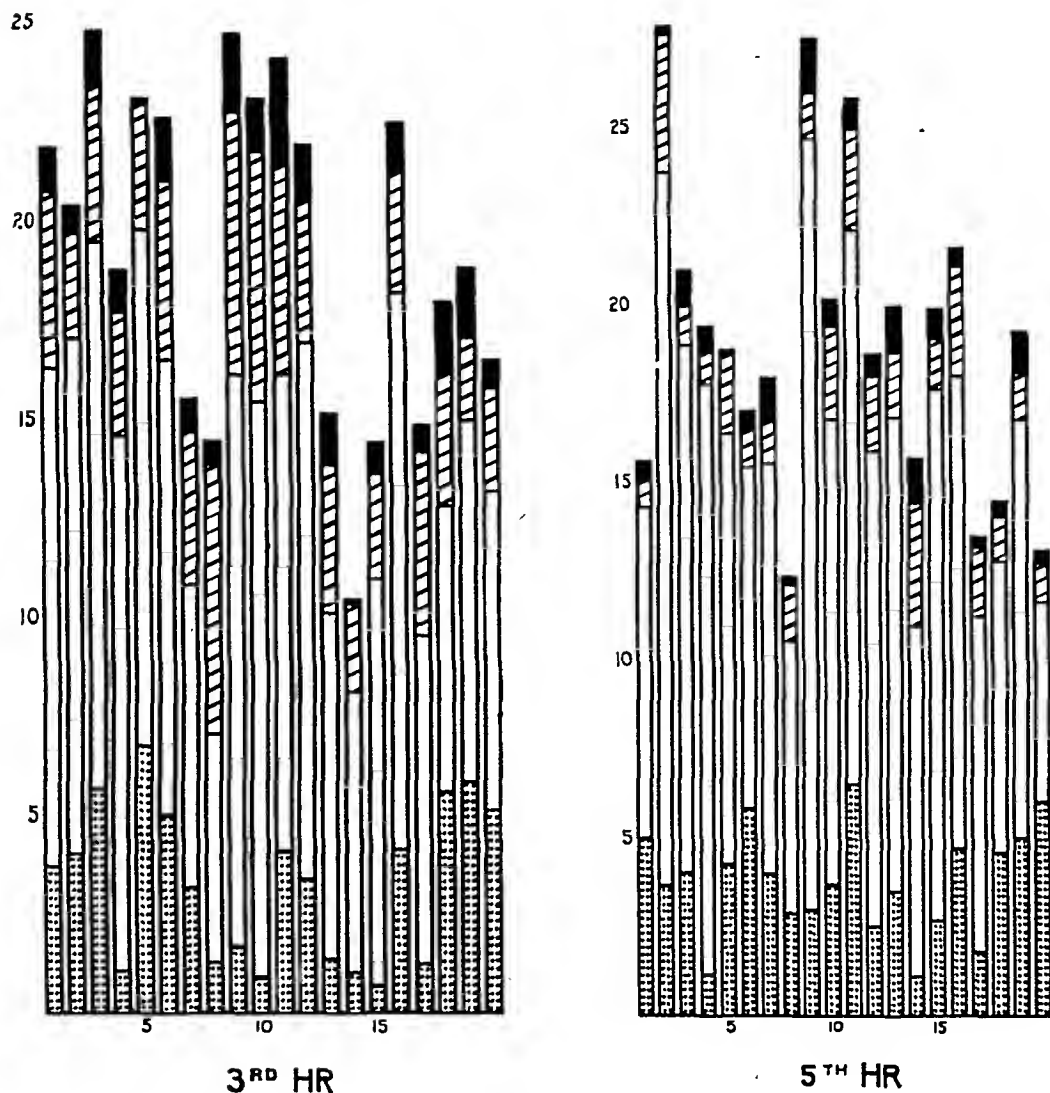


FIG. 1C

Only one dilution was made for the red blood cell counts, but each dilution was checked for technical error by multiple counting. Hemoglobin estimations were made by the acid hematin method of Sahli, and hematocrit values and sedimentation rates were obtained by the Wintrobe methods.

RESULTS

Series I (Colorado Psychopathic Hospital)

A leukocytosis occurred in each case (Figs. 1 and 2), but there was considerable variation in the degree of elevation in individual patients which was in no way

correlated with the amount of insulin given (Table 1). For the group, the average rise in the total count was from 8501 ± 2147 to $21,104 \pm 3769$ or an increase of 148 per cent above their initial values.

The hourly studies demonstrated a general pattern that was characteristic of all the cases and consisted of a gradual elevation of the total count each hour following the administration of insulin to a maximum which appeared either on the third, fourth or fifth hour. After the peak of the rise was attained, a sharp fall in the count usually occurred for the next hour or two, followed by a more

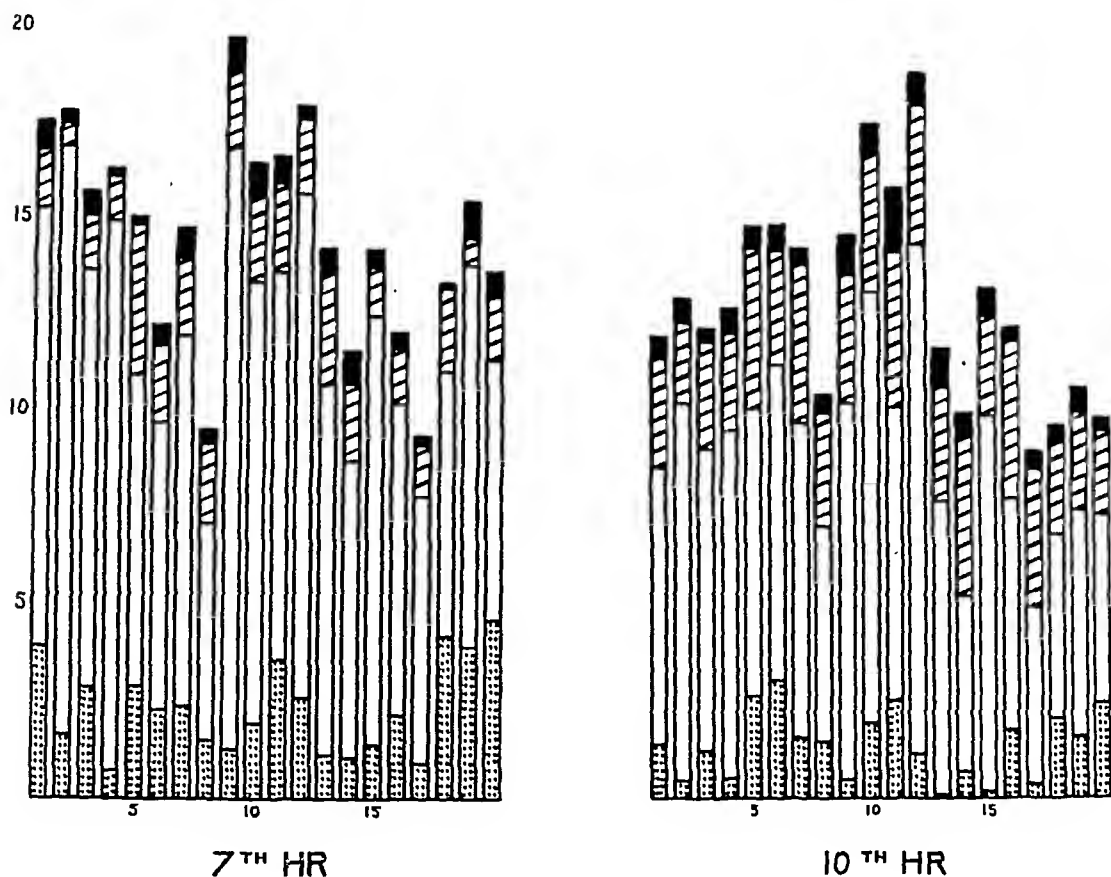


FIG. 1D

gradual hourly decrease with the last count, ten hours after the injection, still considerably above pre-injection levels.

Besides altering the total white cell count, the insulin induced some interesting changes in the differential picture. An absolute neutrophilia, traces of which were present as early as the first hour after insulin, progressively developed to a maximum by the fifth hour; the average rise in absolute numbers was 251 per cent above pre-insulin levels. However, for the first hour or two the percentile values for the polymorphonuclear neutrophils remained essentially normal, after which a relative neutrophilia developed until at the sixth hour after injection of insulin the neutrophilic granulocytes constituted 90 per cent of the total count (Table 4).

With the rise of neutrophils there was found an increasing percentile number of immature forms. The rate of increase of these stab cells even exceeded the rapid

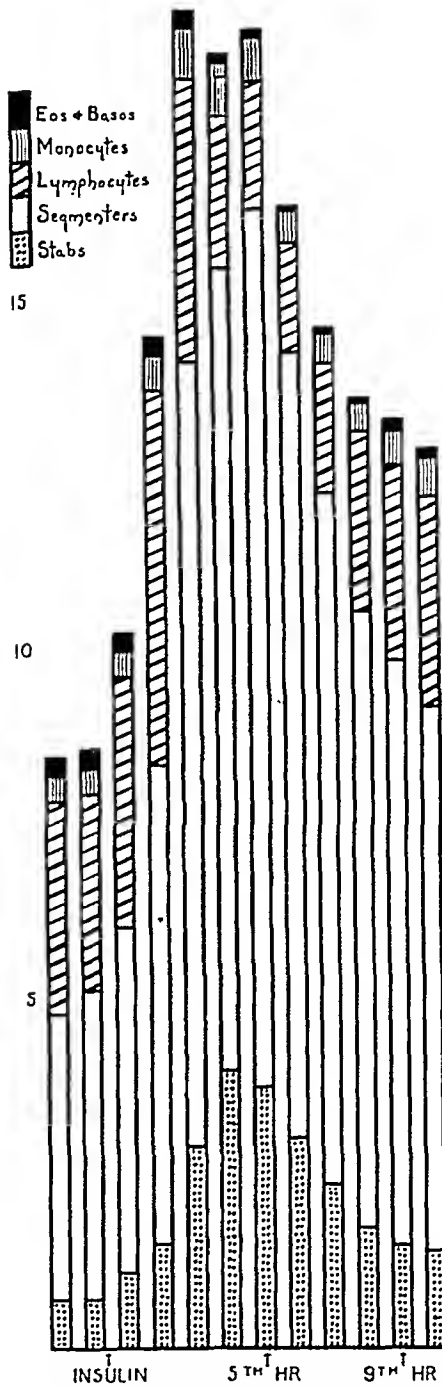


FIG. 2. Hourly averages of the leukocyte counts of 20 patients following injection of insulin in shock therapy (Series I). Values of component cells are shown graphically in absolute numbers in thousands.

rise of the neutrophils to a point where they made up a greater percentage of the elevated neutrophilic values. In addition to revealing increasing immaturity, the neutrophilic granulocytes routinely showed evidences of injury in the form of

toxic granulations. Coincident with the time of the maximum leukocytic rise, the eosinophils and the basophils completely disappeared in most cases, but had a tendency to return to pre-injection normal levels by the tenth hour.

Lymphocytic changes were twofold (Fig. 3). An absolute lymphocytosis occurred as early as the second hour after injection of insulin, that is, before any marked leukocytosis had developed, and at that time the lymphocyte counts showed an average elevation of 81 per cent above their original values. However, by the time the leukocytic rise was the greatest, five hours after insulin, the lymphocytes had decreased to the point of an absolute lymphopenia, averaging only 42 per cent of their original pre-insulin numbers per cu. mm. This phase of absolute lymphopenia lasted only two or three hours and the normal absolute values were re-established by the ninth hour after the administration of insulin.



FIG. 3. Averages of the absolute numbers of lymphocytes in thousands in the 20 patients studied hourly (Series I).

There was an absolute, but not a relative, increase in the numbers of monocytes which coincided in time with the rise of the total count. The monocyte count returned to normal by the sixth or seventh hour in contrast to the slower return of the total count.

Series II (Fitzsimons General Hospital)

Group A (Deep Shock Therapy). As previously shown, the maximum elevation of the total white cell count might occur at either the third, fourth or fifth hour after insulin. Thus, the counts taken in this group only at the fourth hour after injection of insulin would not necessarily represent the maximum leukocytic response of every patient. Regardless of this, the single four hour count demonstrated a leukocytosis in all patients in this group, with the majority exhibiting over 100 per cent elevation (Fig. 4 and Table 2). The fifteen hour count revealed some residual elevation in all but two of the patients, with one-half of all the patients still having a leukocytosis of at least 30 per cent above pre-insulin levels.

The differential picture four hours after insulin was analogous to that seen in Series I at the corresponding time, with a predominance of polymorphonuclear neutrophils and a definite shift to the left. Due to the fact that the four hour count did not represent the time of the maximum leukocytic effort of every individual and that the lymphocytic picture was found to be contingent upon the

time of the maximum rise, the lymphocytes varied from elevated to subnormal values in the individual cases. At the time of the last count (fifteen hours after

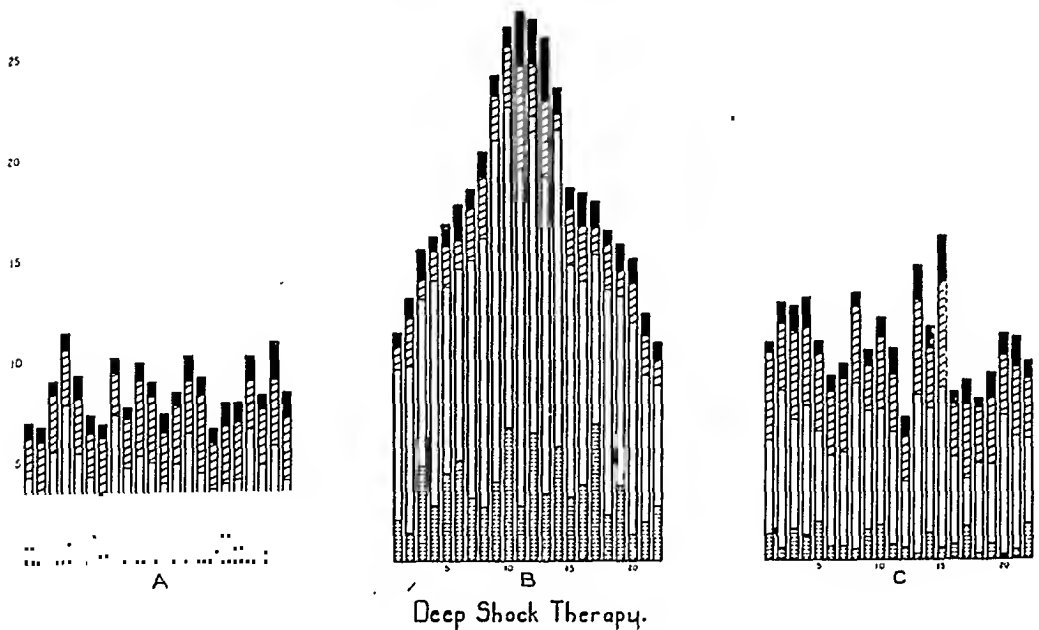


FIG. 4. Series II, Group A (deep shock therapy, Fitzsimons General Hospital). "A" represents counts in thousands prior to insulin; "B", four hours after injection; "C", fifteen hours after injection.

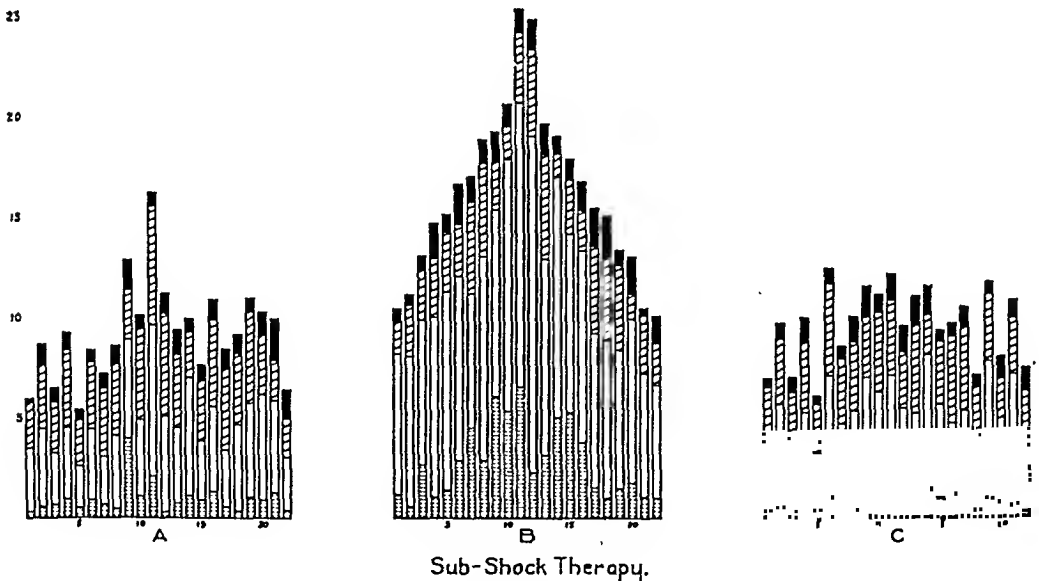


FIG. 5. Series II, Group B (subshock therapy, Fitzsimons General Hospital). "A" represents counts in thousands prior to insulin; "B", four hours after injection of insulin; "C", fifteen hours after injection.

insulin) the differential picture was to a large extent back to normal with the exception of the neutrophils which were still above the initial values.

Comparison of red blood cell counts taken before insulin with those taken four

hours after insulin revealed quite routinely an increase in the erythrocyte count during therapy; this rise averaged 240,000 cells per cū. mm. (Table 2).

Similar comparison of hemoglobin values demonstrated an average rise of 0.55



FIG. 6. The leukocytic picture of a patient followed for thirty consecutive hours, during which two injections of insulin were given. Identical amounts of insulin (120 units) were given at 7:05 a.m. on each day.

Gm. per 100 ml. of blood. In a like manner we found an average increase of two volumes per cent in the hematocrit four hours after insulin.

The sedimentation rates were depressed during therapy by a few millimeters in most of the cases, with an average decrease of 2.7 millimeters in one hour; corrected values, however, showed no alteration.

Group B (Subshock Therapy). A leukocytosis was found in all cases, but to

TABLE 1

SERIES I (COLORADO PSYCHOPATHIC HOSPITAL). A COMPILATION OF THE TOTAL AND DIFFERENTIAL COUNTS FOUND IN EACH OF THE 20 PATIENTS WHO WERE STUDIED HOURLY. THE AVERAGE OF THE TWO COUNTS MADE BEFORE INSULIN IS REPORTED AS THE "INITIAL" COUNT. "Hr." INDICATES THE NUMBER OF HOURS AFTER INSULIN WAS INJECTED. "Ten Hr." INDICATES THE VALUES OBTAINED ON THE LAST DETERMINATION MADE TEN HOURS AFTER INSULIN WAS INJECTED.

CASE NUMBER	NUMBER OF PREVIOUS TREATMENTS	DOSE OF INSULIN	TOTAL LEUKOCYTES				NEUTROPHILS			STAB CELLS		LYMPHOCYTES				MONOCYTES		EOSINOPHILS		BASOPHILS		
			Initial	Maximum		Ten Hr.	Initial	Maximum	Ten Hr.	Initial	Maximum	Maximum		Initial	Maximum	Initial	Fifth Hr.	Initial	Fifth Hr.			
				Number	Hr.							Number	Hr.									
		units																				
1	2	70	5256	21800	3	11950	2385	16698	8568	524	4968	2457	6837	2	730	5	228	1197	192	0	0	
2	21	140	6375	27900	5	12950	4757	23882	10218	808	3627	1353	3711	5	708	7	231	662	33	0	0	
3	12	155	6743	24737	3	12187	3260	19468	9055	756	5615	2907	3958	3	999	6	583	1163	246	63	9	
4	2	65	8225	20000	6	12700	5542	17940	9525	277	1800	2049	4382	2	907	5	582	991	43	0	14	
5	3	60	8056	23006	3	14862	5617	19719	10106	1420	6741	2896	5207	2	748	4	373	145	97	0	47	
6	42	95	9243	22500	3	14887	5863	16425	11259	1617	4950	2486	4981	2	1065	5	336	900	479	169	79	
7	32	130	9443	17825	5	14262	4722	15454	9698	755	4353	4013	5143	2	1248	5	472	1069	169	0	61	
8	19	200	9850	12550	4	10425	4995	10471	7016	833	2823	4360	6797	3	1559	5	221	502	253	123	30	
9	4	75	10675	27400	5	14600	6707	24578	10220	144	2032	3167	9998	2	1288	5	567	1562	160	0	75	
10	17	170	13725	22950	3	17500	7942	16702	13150	631	3609	4767	7632	2	2240	7	742	1308	161	60	114	
11	14	75	11968	25662	5	15812	6130	21992	10120	651	6415	4527	5979	2	1220	6	758	1982	435	180	118	
12	17	45	10000	21800	3	18825	6300	17724	14363	882	3447	2914	6001	2	1442	6	362	958	330	55	0	
13	38	100	9488	19825	5	11675	4750	16712	7706	249	3429	3424	3839	4	1841	5	365	852	712	258	111	
14	1	45	9225	15550	5	9950	5167	10885	5204	319	1526	3412	5922	1	1890	4	411	1088	383	109	27	
15	24	290	9228	20050	4	13225	6512	17158	9919	332	3010	2100	4521	2	1274	7	397	1063	120	0	91	
16	23	115	7588	22337	3	12212	4635	18092	7730	565	4657	2213	5559	2	1437	7	414	1005	297	0	29	
17	18	75	6575	18500	4	8975	2740	14375	4909	232	2646	3399	6079	2	1320	7	266	685	112	40	55	
18	17	155	6506	22137	4	9675	4046	17555	6840	651	5911	2052	3426	2	1247	5	292	1193	111	155	10	
19	16	150	6356	19137	5	10662	3230	16649	7463	984	5785	2587	3492	1	576	6	158	1362	254	134	126	
20	12	150	5487	16375	3	9837	3136	13051	7348	1027	6140	1854	3384	2	967	6	230	875	239	39	28	
Average.....	17	118	8501	21104	4	12859	4922	17277	9021	685	4219	2947	5343	2	1235	6	399	1039	219	69	53	
Standard deviation...	±11	±59	±2147	±3769		±2606	±1431	±3534	±2307	±381	±1517	±917	±1622		±417		±167	±347	±161	±76	±45	±32

a slightly less extent than was found in patients with deep shock, since not quite one-half of the patients exhibited a rise of over 100 per cent (Fig. 5 and Table 3). In conjunction with this lessened elevation of the total count there was a greater tendency to return to normal in fifteen hours, evidenced by normal counts in one-half the patients, and only slight elevations in the remaining patients at that time.

TABLE 2

SERIES II, GROUP A (DEEP SHOCK THERAPY FITZSIMONS GENERAL HOSPITAL).

"A" SIGNIFIES PRIOR TO INJECTION OF INSULIN; "B", FOUR HOURS AFTER INJECTION; "C", FIFTEEN HOURS AFTER INJECTION

CASE NUMBER	DOSE OF INSULIN	TOTAL LEUKOCYTE COUNT			ERYTHROCYTES		HEMOGLOBIN		HEMATOCRIT (WINTROBE)		SEDIMENTATION RATE (WINTROBE)	
		A	B	C	A	B	A	B				
	units				millions		Grams				mm. in 1 hr.	
1	140	6987	11317	10762	4.30	5.30	15.9	16.2	45.0	49.5	6.0	5.5
2	120	6750	13050	12700	4.78	5.10	14.5	14.8	48.0	50.0	10.0	8.0
3	80	9062	15433	12537	4.83	5.15	15.8	16.0	46.5	47.5	12.0	9.0
4	110	11375	16037	12925	4.35	4.78	13.2	13.4	50.5	51.5	3.0	4.0
5	140	9300	16712	10762	5.25	5.80	17.1	16.8	50.5	51.5	1.5	1.0
6	90	7300	17625	9087	4.61	5.14	13.2	14.5	52.0	51.5	9.0	7.5
7	240	6850	18350	9650	5.49	5.81	15.4	15.0	52.0	55.0	1.5	0.5
8	150	10150	20200	13150	4.70	5.21	14.4	15.1	48.0	50.5	20.0	8.0
9	80	7700	23975	10325	4.90	5.15	14.6	15.0	47.0	51.5	16.0	17.0
10	140	9900	26350	11950	4.56	5.15	14.5	14.1	48.0	51.5	16.0	4.5
11	150	8750	27125	10450	5.34	5.07	13.9	12.9	50.0	48.0	9.0	11.0
12	140	7387	26762	7000	4.83	4.80	13.7	13.8	48.5	49.0	4.5	4.5
13	200	8475	25825	14500	4.84	4.70	15.9	17.0	48.5	53.5	18.0	3.0
14	140	10293	23317	11525	4.80	5.22	15.5	15.8	48.0	50.0	9.5	6.0
15	100	9200	18400	15975	5.06	4.60	13.3	13.7	48.0	50.0	9.0	10.0
16	170	6625	18175	8225	5.08	5.20	15.1	16.0	51.0	54.0	15.0	3.5
17	100	7912	17712	8637	4.25	4.38	15.1	17.0	44.5	51.0	9.0	8.0
18	50	7900	16287	7899	4.36	5.01	14.9	15.4	45.0	48.0	5.5	5.5
19	230	10250	15633	9162	5.27	4.80	13.2	13.1	44.5	44.5	38.0	38.0
20	140	8325	14925	11125	4.59	5.19	13.2	13.9	50.0	51.5	8.0	8.0
21	220	10950	12200	10962	5.70	5.52	17.0	17.2	52.0	54.0	2.0	2.0
22	110	8438	10687	9750	4.97	5.70	16.0	16.7	48.0	52.0	3.0	1.5
Average.....	138	8631	18459	10865	4.84	5.08	14.7	15.3	48.4	50.7	10.2	7.5
Standard deviation..	±49	±1382	±4964	±2149	±0.46	±0.44	±2.0	±1.8	±2.9	±2.4	±8.1	±7.6

The differential picture after insulin was similar to that seen in Group A.

The red blood cell counts, hemoglobin and hematocrit determinations were increased four hours after insulin in amounts comparable to the increases noted in Group A (Table 3).

DISCUSSION

From the results given it is apparent that regular insulin given to fasting patients invariably provoked a leukocytosis within one hour after its adminis-

tration. The leukocytosis was progressive for about four hours, after which it began to disappear, but evidences of it invariably remained for ten hours and frequently for fifteen hours. However, since the counts taken before the administration of insulin were within the normal range, and these patients had been

TABLE 3

SERIES II, GROUP B (SUBSHOCK THERAPY, FITZSIMONS GENERAL HOSPITAL). "A" SIGNIFIES VALUES PRIOR TO INJECTION OF INSULIN; "B", FOUR HOURS AFTER INJECTION; "C", FIFTEEN HOURS AFTER INJECTION.

CASE NUMBER	DOSE OF INSULIN	TOTAL LEUKOCYTES			ERYTHROCYTES		HEMOGLOBIN		HEMATOCRIT (WINTROBE)		SEDIMENTATION RATE (WINTROBE)	
		A	B	C	A	B	A	B	A	B	A	B
	<i>units</i>				<i>million</i>		<i>Grams</i>				<i>mm. in 1 hr.</i>	
1	20	5975	10380	6967	4.55	4.81	16.8	17.0	45.0	47.0	6.5	3.5
2	80	8700	11100	9625	5.17	5.23	15.0	15.2	50.5	51.5	3.5	3.0
3	80	6475	12975	6925	4.73	5.15	12.9	13.2	45.0	48.0	13.0	9.0
4	30	9237	14587	9887	5.55	5.70	17.5	17.8	52.5	54.0	5.0	6.5
5	50	5360	15050	6000	3.80	4.20	13.2	12.8	41.0	43.5	13.0	14.0
6	50	8350	16525	12300	5.23	4.98	13.5	14.2	47.0	49.0	13.0	11.0
7	60	7150	16900	8480	4.53	4.30	15.4	14.8	50.0	49.5	7.5	8.0
8	80	8550	18750	9950	4.77	4.85	12.0	12.2	47.0	49.0	6.0	6.0
9	30	12850	19137	11450	4.67	4.98	15.2	16.8	46.5	48.0	9.0	4.5
10	50	10063	20510	11033	4.95	4.82	14.2	14.8	50.0	51.0	3.0	3.0
11	30	16200	25125	12025	4.43	4.76	13.2	14.0	48.0	48.5	5.0	6.5
12	90	11175	24625	9500	4.98	5.06	14.0	14.5	49.5	50.0	6.0	5.0
13	30	9325	19500	10950	4.94	4.90	14.4	14.4	49.0	50.0	8.0	8.0
14	90	9862	18837	11062	4.79	5.55	14.2	16.3	50.5	54.5	8.0	2.5
15	50	7570	17737	9510	4.78	5.20	14.6	14.8	49.0	50.5	2.0	3.0
16	40	10881	16612	9650	4.54	4.50	16.4	17.0	47.5	50.0	6.0	5.5
17	50	8375	15387	10450	4.79	5.42	12.5	14.5	50.0	54.5	7.0	3.0
18	30	9100	14900	7025	4.55	5.07	12.8	13.6	45.4	47.0	8.5	8.0
19	60	10825	13237	11750	4.22	4.50	13.0	12.7	47.5	49.0	5.0	6.0
20	30	10237	12825	7983	5.62	5.65	16.0	16.0	54.5	55.0	0.5	0.5
21	50	9900	10362	10775	4.97	5.36	14.4	15.9	51.5	52.5	5.0	3.5
22	70	6350	9937	7437	5.30	5.54	16.0	16.2	48.5	50.5	9.0	3.5
Average.....	52	9205	16136	9575	4.81	5.02	14.4	14.9	48.4	50.1	6.8	5.6
Standard deviation..	±21	±2375	±4130	±1806	±0.41	±0.41	±1.5	±1.5	±2.9	±2.9	±3.2	±3.0

receiving treatment the previous day, it is obvious that the leukocytosis induced by insulin disappeared within twenty-four hours.

In general, we noted no correlation between the degree of leukocytosis and the amount of insulin given. This is perhaps best illustrated by the variation in the leukocytic response to identical amounts of insulin in one patient on whom hourly counts were made during two consecutive days of treatment (Fig. 6).

Likewise, we noted no exhaustion of the patient's ability to produce a leuko-

cytosis because of previous daily injections of insulin, since the degree of leukocytosis was apparently as great after forty days of treatment as during the first few days (Table 1, Cases 4 and 6).

In the differential picture an amazing similarity existed between leukocytosis induced by insulin and that produced by an acute pyogenic infection. Not only did each cause a marked neutrophilia with a shift to the left and the appearances of toxic granulations, but each also demonstrated a lymphopenia at the time of the maximum leukocytosis in conjunction with a disappearance of the eosinophilic and basophilic granulocytes. However, we observed two points of variance in the leukocytosis following insulin that afforded a means of distinguishing it

TABLE 4

SERIES I. A COMPOSITE PICTURE OF THE 20 PATIENTS STUDIED HOURLY, SHOWING IN ABSOLUTE NUMBERS AND PERCENTAGES THE COMPONENT CELLS AT EACH HOUR

TIME OF COUNTS	TOTAL COUNT	NEUTROPHILS		STAB CELLS		LYMPHOCYTES		MONOCYTES		EOSINOPHILS		BASOPHILS	
		Absolute Numbers	Per cent	Absolute Numbers	Per cent	Absolute Numbers	Per cent	Absolute Numbers	Per cent	Absolute Numbers	Per cent	Absolute Numbers	Per cent
6 a.m.	8460	4785	56.56	675	7.97	2999	35.44	388	4.58	241	2.85	51	0.60
7 a.m.	8578	5109	59.55	684	7.97	2818	32.85	412	4.80	229	2.67	51	0.59
7:05 a.m.	Insulin administered												
8 a.m.	10221	6028	58.97	1067	10.46	3581	35.03	345	3.38	226	2.21	45	0.44
9 a.m.	14479	8352	57.61	1461	10.09	5208	35.90	549	3.79	218	1.51	69	0.48
10 a.m.	19179	14138	73.71	2884	15.03	3927	20.47	797	4.15	242	1.26	55	0.28
11 a.m.	18536	15472	83.47	3961	21.37	2175	11.74	765	4.13	65	0.35	38	0.21
12 Noon	18861	16311	86.48	3736	19.80	1861	9.86	621	3.30	69	0.37	14	0.08
1 p.m.	16344	14259	87.24	2995	18.32	1571	9.61	463	2.83	46	0.28	17	0.11
2 p.m.	14593	12231	89.30	2316	15.87	1822	12.48	470	3.22	31	0.22	30	0.21
3 p.m.	13577	10541	77.80	1703	12.54	2549	18.77	416	3.07	36	0.27	28	0.21
4 p.m.	13261	9823	71.81	1458	10.99	2935	22.13	470	3.55	89	0.67	31	0.24
5 p.m.	12856	9158	71.23	1378	10.71	3182	24.75	538	4.18	95	0.74	38	0.30

from the pyrogenically produced type: concomitant with the insulin induced leukocytosis there was no such alteration in the sedimentation rate as is usually observed in a pyogenic leukocytosis; and, secondly, while a bacterially engendered leukocytosis usually lasts for several days, that produced by insulin is decidedly ephemeral.

Leukocytosis may be considered to be produced by one or any combination of three processes:

1. Dehydration, when excessive and prolonged.
2. Mobilization of leukocytes from stored spaces, such as may occur when collapsed capillaries are washed out by an increased blood flow.
3. Increased rate of production as the result of bone marrow stimulation.

Dehydration quite obviously must occur during insulin therapy, particularly in the wet shock cases, since there is profuse sweating with no fluid intake for

more than four hours. The slight increase in the red blood cell count, found in most cases during this period, can probably be explained on this basis.

The degree of leukocytosis engendered by dehydration can be ascertained by inductive logic, if we assume that the entire rise in the red blood cells is the result of dehydration. The ratio of the erythrocytes to the leukocytes of the patients in Series II was approximately 550 to 1 prior to treatment, and would not be altered by simple dehydration. The average erythrocytic increase in this group during treatment was 240,000 cells per cu. mm., which on an equivalent basis would increase the leukocytes 435 cells per cu. mm., an amount within the range of error in counting.

Since dehydration can play only a small part in this leukocytosis, mobilization or bone marrow stimulation, or both, may be considered as the main factor or factors. Subsequent bone marrow studies may help to elucidate the mechanism.

SUMMARY AND CONCLUSION

The administration of regular insulin in 64 mental patients resulted in alterations of the blood picture.

A leukocytosis occurred in all patients with the peak elevation occurring between the third and fifth hour, after which there was a sharp fall for two or three hours followed by a more gradual decline, with elevated values still demonstrated fifteen hours postinsulin. Normal values were obtained in twenty-four hours.

The rise in the total white count was largely neutrophilic.

The eosinophils and the basophils had a tendency to disappear at the time of the maximum leukocytic rise.

The lymphocytes were changed in percentage and absolute numbers with a lymphocytosis developing by the second hour, following which they were gradually depressed to the point of an absolute lymphopenia by the sixth hour. Normal values were again established by the ninth hour after injection of insulin.

There was a slight rise in the red blood cells, hemoglobin and hematocrit values following the injection of insulin, as shown in the counts taken four hours after its administration.

The sedimentation rate was essentially unaltered, and may serve as a means of differentiating an insulin produced leukocytosis from the leukocytosis of infection.

It is obvious from the above findings that the white blood cell and differential counts can not be used as diagnostic adjuncts as evidences of infection, on the day regular insulin is used for shock therapy.

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A CONSIDERATION OF THE USE OF BLOOD AND OXYGEN AS SUPPORTIVE THERAPY IN THE TREATMENT OF MALARIA*

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Clinical and experimental data have been accumulating during the past six years supporting the opinion expressed in 1941 that anoxia is a significant factor in acute types of human malaria. This anoxia results from the rapid destruction of red blood cells by the plasmodia.⁴ A decrease in the number of red cells, accompanied by a diminution in hemoglobin, is a characteristic feature of all types of acute malaria.¹

Acute malaria has been studied extensively in the duck, and it has been found that the oxygen-carrying ability of the blood is reduced to from 15 to 20 per cent of the normal in moribund birds.⁵ Furthermore, the amount of the decrease is proportional to the severity of the infection. Accompanying this fall in oxygen capacity during the course of the disease, there is an acidosis which decreases further the oxygen-carrying capacity of the blood. In man acute malaria is accompanied by an elevated temperature, and this adds an additional strain on the oxygen transportation system and contributes indirectly to the anoxia produced by the acute anemia.⁵ Wong has shown that a decrease occurs in arterial saturation *in vivo* in neurosyphilitic patients undergoing malarial fever therapy.¹¹

Since there is a rapid destruction of red cells in malaria with a resulting anoxia, a replacement of the cells by transfusions should be beneficial to the host. Both clinically and experimentally this has been observed to occur. Ducks with acute malarial infections given multiple transfusions of duck blood show definite clinical improvement during the time the red blood cells counts are kept high.⁶ Monkeys infected with a virulent strain of malaria likewise show clinical improvement when given transfusions of human blood. Observations at this time suggest that probably the beneficial effects resulting from the transfusing of human blood does not result only from the replacement of red cells, but also that human serum produces antibodies in the monkey that are detrimental to *Plasmodium knowlesi*.

Makower gave multiple transfusions to two Polish soldiers with chronic *Plasmodium vivax* infection who were resistant to the action of both quinine and atabrine. He observed a "drastic effect on the course of the disease. The temperature became normal, and the general condition was rapidly improved. However, malaria parasites remained in the blood; in other words, the manifest

* Received for publication, November 7, 1947. The experimental studies on which this paper is based were made at the School of Medicine, University of Arkansas. This work was supported by a grant from the John and Mary R. Markle Foundation. The oxygen was supplied by the Linde Air Products Company, New York, New York. The blood was obtained through the kindness of Mr. Taylor in the Blood Bank at the University of Arkansas.

disease was transformed into a latent form. After the administration of atabrine and plasmochine in the first case and atabrine to the second patient, blood was rendered parasite free."²

The problem of acute anoxia in malaria has been approached therapeutically, both experimentally and clinically, from another viewpoint, that is, by the administration of oxygen. The concentration of oxygen within the lung will influence the amount of oxyhemoglobin in the red cells. When there is a decrease in the number of red blood cells, the tissues suffer from anoxia; however, if the concentration of oxygen is increased in the lungs, this tissue anoxia may be compensated for either partially or completely.

To demonstrate the role of anoxia in malarial infections, ducks were placed in a decompression chamber. The time required for death to occur was proportional to the severity of the infection, and death always occurred sooner in the group in the decompression chamber than in the controls kept at normal atmospheric pressure.⁵ Furthermore, ducks with a severe malarial infection, when placed in an oxygen chamber, showed definite clinical improvement within a period of from fifteen to thirty minutes. The length of life of infected ducks was prolonged from eighteen to twenty-four hours by placing them in an oxygen chamber at an oxygen concentration of from 75 to 90 per cent. Most birds with a severe malarial infection after being in the oxygen chamber died immediately after removal from the chamber. Infected birds kept for long periods in the oxygen chamber showed an increase in the degree of parasitemia.⁷

The observation on the effect of oxygen on malaria in the duck was repeated using the monkey. *P. knowlesi* in the rhesus monkey produced an acute infection characterized by a severe anemia. Treatment of these monkeys with oxygen was instituted when the animals were in different stages of the infection. The clinical response of the monkey to an increased concentration of oxygen was most spectacular. These results are illustrated by monkey number 1. This animal was inoculated with *P. knowlesi*, and the number of red blood cells gradually decreased while the number of parasitized cells increased (Fig. 1). On the morning of the eleventh day of the experiment this monkey was pale, weak and considered to be very sick. During the afternoon the circulation was so poor in the ear that blood could not be obtained from this site for a red blood cell count. At six o'clock on the eleventh day this monkey was still very sick. On the morning of the twelfth he had difficulty in walking and was motionless while blood was being taken for a cell count. At this time he was put into the oxygen chamber that had a concentration of approximately 50 per cent oxygen. Ten hours later he was eating carrots and looked the same as he had twenty-four to thirty-six hours earlier. By noon he had eaten all the carrots fed to him for the morning meal. A red cell count was made at 6:00 P.M. He offered considerable resistance for the first minute while being caught. However, he collapsed within two minutes after the oxygen chamber was opened. He was absolutely motionless during the time blood was being collected for the count. Thirty minutes after being replaced in the oxygen chamber he was sitting up with a piece of bread in his front feet. Four hours later he was sick, but looked

about the same as he did on the preceding day. The oxygen supply was exhausted about 6:30 A.M. on the thirtieth day, and the monkey died shortly thereafter.

The combination of oxygen and quinine in the treatment of monkeys with malaria frequently proved to be beneficial as shown by the following protocol. On the morning of the ninth experimental day the red blood cell count was 1,380,000. There were 324 parasitized cells per 1000 red blood cells. The monkey was so weak that I caught him and gave him 2.0 grains of quinine dihydrochloride intravenously without any assistance. He was put into the oxygen chamber that had a concentration of approximately 40 per cent oxygen. Improvement occurred rapidly, and he ate peanuts and carrots. During the

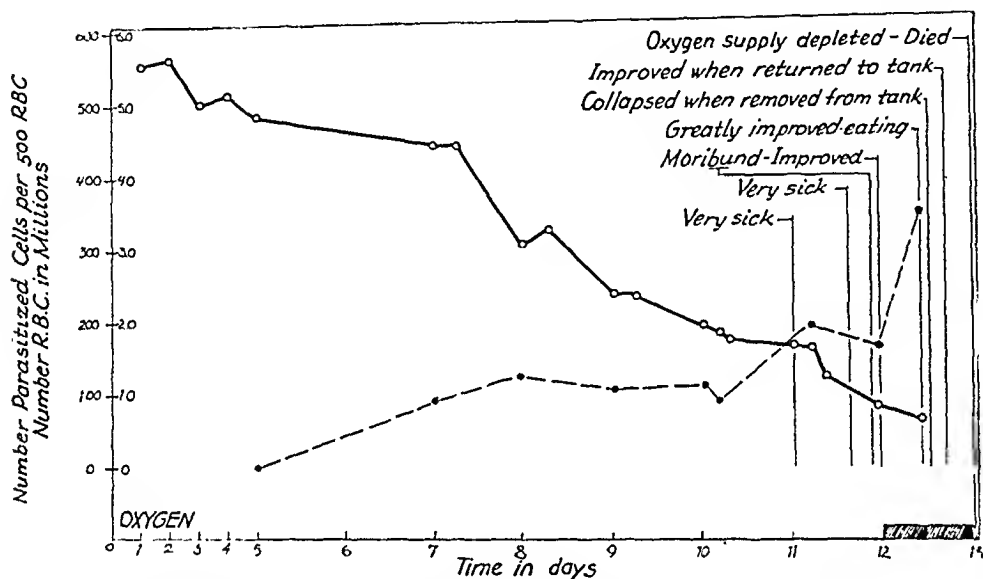


FIG. 1. Monkey 1. This indicates the temporary improvement that occurs when monkeys with a severe malarial infection are put into an oxygen chamber. The length of life was doubtless prolonged by the oxygen.

afternoon and evening of the ninth day, this monkey appeared to be in very good health. At 7:30 A.M. on the tenth day he was moribund and died fifteen minutes later (Fig. 2). Had we been interested in saving this animal, a second dose of quinine would have been given during the afternoon of the ninth day. One cannot predict whether the animal would have survived the infection, but there can be no question that improvement followed immediately upon the use of oxygen.

A third group of experiments was performed on monkeys with malaria. These were given quinine, transfusions of human blood and kept within an oxygen chamber. Monkey number 3 illustrates the effect of this combination of therapeutic agents (Fig. 3). On the tenth experimental day the red blood cell count was 2,080,000, and there were 179 parasitized cells per 1000 red blood cells. On the eleventh experimental day this monkey was so sick that I caught him without any assistance and gave him 1.5 grains of quinine dihydrochloride

intravenously and 60 cc. of type O human syphilitic blood intraperitoneally. He was put immediately into the oxygen chamber that had a concentration of approximately 40 per cent oxygen. This monkey was as near death

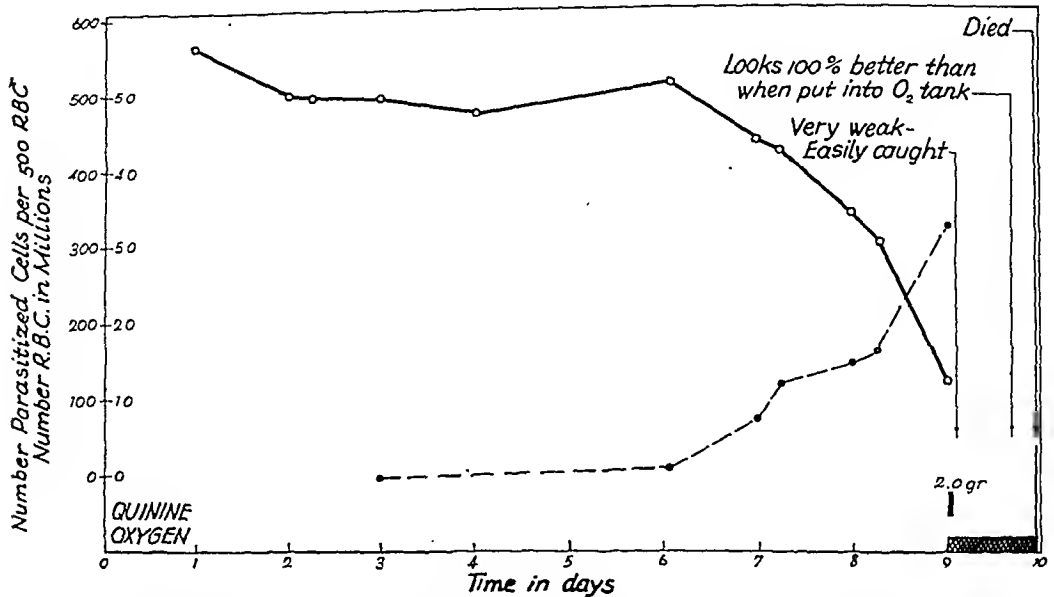


FIG. 2. Monkeys 131. The combination of quinine and oxygen is beneficial to the monkey. The oxygen may serve as a supportive measure during the interval before the quinine reaches a concentration sufficient to destroy the plasmodia.

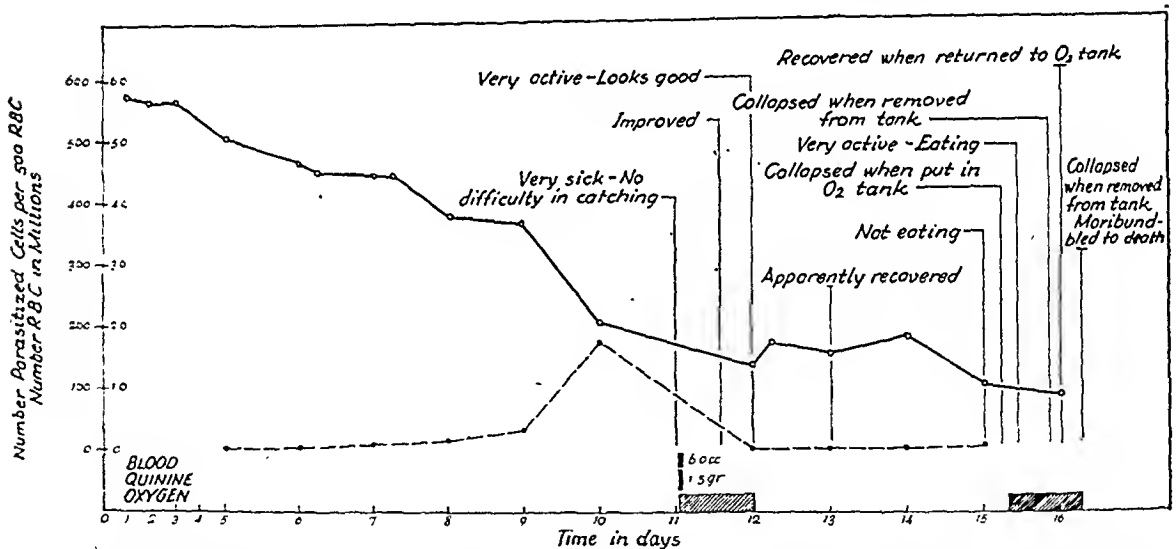


FIG. 3. Monkey 3. The use of blood and oxygen as supportive measures in the treatment of acute malarial infections in the monkey is beneficial. This animal was as near death as any we studied. At the time he was given 1.5 grains quinine, 60 cc. blood and put into the oxygen chamber. Additional quinine would have been given if we had wished to prevent the death of this monkey.

as any we studied in these experiments. He improved during the day, and on the following morning he looked well and was very active. The red blood cell count was 1,390,000, and one parasitized cell was found in 1000 red blood cells. For three days this monkey appeared to be normal. At this time

the number of red blood cells began to decrease and the parasitemia to increase. Death occurred on the afternoon of the sixteenth day of the experiment. From our experience with this strain of malaria we would say that this monkey was greatly benefited by the use of the oxygen, blood and quinine. It is questionable whether survival would have occurred if only quinine had been given during the time of the first acute attack.

Oxygen has been used in the treatment of human cases of malaria apparently more frequently than blood transfusions. Trupp¹⁰ states that oxygen alone has been used in a small number of cases and the results were at times startling. The Russians consider that supportive therapy such as oxygen is advisable in the treatment of fulminating forms of tertian malaria.⁹

The rationale for the use of transfusions and oxygen in the treatment of acute forms of malaria appears to be based upon sound physiologic principles. As previously stated, there is destruction of red blood cells by the plasmodia. With a decreased number of circulating red cells, the tissues suffer from anemic anoxia. Accompanying this anemic anoxia, there is also the development of stagnant anoxia which may lead to increased permeability of capillaries with the escape of fluids into the tissues. If this type of anoxia is not relieved, hemoconcentration occurs, the capillaries may become occluded and petechiae may result. This apparently is the explanation for the hemorrhages that so frequently occur in *Plasmodium falciparum* infections. Why the clinical manifestations in some cases of this infection are referable to the brain, the gastrointestinal tract, the lungs or the heart is not known at this time.

Trupp¹⁰ in 1946 discussed the problem of "metabolism in cerebral malaria" and emphasized the necessity of combating the anoxia. He expressed the opinion that oxygen therapy alone has a tendency to result in constriction of small arterioles and thereby decreases cerebral blood flow. Since nicotinic acid increases cerebral blood flow and aids in restoration of respiratory systems, Trupp has considered the use of a combination of oxygen and nicotinic acid in the treatment of *P. falciparum* infections. Three cases of cerebral malaria are reported by Trupp in which 100 mg. of nicotinic acid was given every four hours for two days. Oxygen was given almost continuously for thirty-six hours with a B.L.B. (Boothby, Lovelace and Bulhilian) oxygen mask to 1 patient, and the other 2 were given oxygen for one hour out of each four during a period of two days. Each patient experienced rapid relief from symptoms and made an uneventful recovery.

Most and Meleney³ in 1944 reported a case of cerebral malaria treated by the intravenous injection of nicotinic acid. Consciousness was restored immediately. The use of nicotinic-acid-amide has reduced markedly the morbidity of uncomplicated falciparum malaria.¹⁰ Nicotinic acid may also reduce the toxicity of quinine when it is used in large doses or when a debilitated patient appears excessively sensitive to quinine.¹⁰

The use of transfusions, oxygen and nicotinic acid are only adjuncts to specific therapy in the treatment of malaria. Supportive therapy may keep a patient alive during the interval necessary for the plasmodicidal agent to produce its

specific effects. Several monkeys with acute malaria have been treated with a combination of blood, oxygen and quinine. Although the time at which therapy was instituted apparently was too late in some of the animals, others responded excellently to the regime.

SUMMARY

The use of transfusions and oxygen as supportive measures to combat anoxia, together with specific therapeutic agents for the treatment of acute forms of malaria, is based upon sound physiologic principles. Furthermore, pathologic studies indicate that anoxia is a significant process in the mechanism of death in cases of acute malaria both in man and in experimental animals. As far as is known, there are no contraindications to the use of blood and oxygen in therapeutic doses in cases of malaria. The observation that the parasitemia increases in ducks and probably in the monkey when the hosts are kept for a long time in the presence of a high concentration of oxygen, is not significant in the treatment of human infections since plasmodicidal drugs are also given at the same time as the blood and oxygen.

The additional use of nicotinic acid to facilitate the enzyme system in the treatment of malaria appears to be acceptable therapy, and it is recommended.

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THE CLINICAL EVALUATION OF HYALURONIDASE IN HUMAN INFERTILITY*

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In a previous communication,² the enzyme hyaluronidase was shown to be of value in the treatment of human infertility. The present paper is an extension of this work.

Karl Meyer⁹ has recently presented a review of the relationship of the enzyme to its substrate, hyaluronic acid. Hyaluronic acid is a mucopolysaccharide acid which, in animal tissues, seems to bind water in interstitial spaces. It further holds cells together in a jelly-like matrix and serves as a lubricant and shock absorber in joints. It is disaggregated and depolymerized by the action of the enzyme hyaluronidase. Hyaluronic acid is of unknown structure. It is composed of an equimolar combination of glucuronic acid and n-acetylglucosamine and is polymerized to very large and elongated molecules having a molecular weight of from 200,000 to 500,000.⁸ Solutions are consequently highly viscous, as little as 0.1 per cent in saline being twelve times as viscous as water.⁷ When hyaluronate is hydrolyzed by hyaluronidase, the polysaccharide is first depolymerized and then hydrolyzed. The reactions characteristic of the intact polymer disappear; first, there is lost the property of forming a turbid sol with protein and of forming a clot; then, the viscosity of the solution falls. The analytic procedures to determine the action of hyaluronidase on hyaluronate depend on this sequence of events. The test for hyaluronidase in semen, as carried out in our laboratory, is based on the fact that the addition of hyaluronic acid to acidified protein (blood serum) produces a visible precipitate or ring of "mucin". In the presence of hyaluronidase the formation of a mucin clot is prevented owing to degradation of hyaluronic acid by the enzyme.

Hyaluronidase occurs in numerous bacteria and in a large variety of organs and tissues, including neoplasms. It bears a close similarity, if not identity, to the spreading factors. Hyaluronidase seems to be implicated in diverse biologic phenomena, including fertilization of the ovum.

Two significant facts are evident in fertilization, namely: (1) The viscous gel in which the follicle cells of the *cumulus oophorus* are imbedded contains, or is most likely composed of, a hyaluronic acid complex. (2) The testicle contains a great concentration of hyaluronidase. The implications inherent in these facts led McClean and Rowlands⁶ to demonstrate the essential role of hyaluronidase in fertilization of the mammalian ovum.

Pincus and Enzmann¹¹ demonstrated that a heat-labile enzyme is present in spermatozoa which disintegrates the follicular complex of the ovum. McClean and Rowlands⁶ made the interesting observation that hyaluronidase is capable of dis-

* Presented at the Twenty-Sixth Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 28, 1947. Received for publication, October 28, 1947.

persing the follicle cells which surround the *recently ovulated* mammalian ovum. The follicle cells that surround the mammalian ovum are embedded in a transparent viscous gel which must be removed before the sperm can penetrate the egg. Hyaluronidase prepared from various sources (testes, bacteria, snake venom) was allowed to act on the recently ovulated ova. The follicle cells were dispersed without dissolution until the egg was left completely denuded. The enzyme action was found to be restricted apparently to the liquefaction of the gel without affecting the ovum itself. McClean and Rowlands pointed out that these observations could account for certain types of sterility as being due either to an insufficient concentration of hyaluronidase, as in instances of a low sperm count, or else to an actual deficiency in the formation of the enzyme by the male organism. Fekete and Duran-Reynals¹ confirmed the dispersing effect of hyaluronidase on the follicle cells surrounding the mouse ovum, and Leonard and Kurzrok² corroborated the observations on rat ova (Figs. 1 and 2).

Leonard, Perlman and Kurzrok⁴ noted that the enzyme papain dispersed the follicle cells with equal rapidity, but at the same time seemed to damage the ovum. They suggested a rat ova test for the biologic standardization of hyaluronidase. A rat ova unit of hyaluronidase is defined as the amount of enzyme in 0.2 cc. of Ringer's solution which will disperse the follicle cells of two out of three or one-half of a greater number of ova not exceeding eight, within three-fourths to one hour. The follicle cells are considered dispersed when not more than a single layer of cells surrounds an ovum. It was found that with the purest preparation of hyaluronidase available, 33 gamma of enzyme was necessary in order to disperse the follicle cells in the rat *in vitro* under standard conditions. In the course of some experiments upon the determination of this enzyme in the testis and in semen, it was observed that the concentration in the ejaculate of the rat, obtained from the uterus of the female shortly after breeding, was inordinately high as compared with the enzyme concentration from extracts of rat testis. It was considered possible that the secretions of the prostate and seminal vesicles might favor the production of hyaluronidase by furnishing a better environment for the continued production of enzyme. Experiments demonstrated that the addition of rat seminal vesicle tissue to rat testis homogenate resulted in an increased production of hyaluronidase as compared with testis homogenate alone. Prostatic tissue did not exhibit this effect, although both prostate and seminal vesicles normally contain hyaluronidase. It is thought that this hyaluronidase diffuses into these organs. It was not possible to detect any enzyme in prostatic tissue obtained from castrate immature rats treated with testosterone and in which, of course, no spermatozoa were ever present.

The finding that the addition of seminal vesicle tissue to the testis homogenate greatly increased the production of hyaluronidase, complements the work of Mann⁵ who has shown that the primary energy source for sperm is fructose. This sugar was found to occur in its highest concentration in the seminal vesicles or prostate, according to the mammalian species. Therefore, it would seem that one of the functions of the seminal vesicle secretion is to increase the hyaluronidase output of spermatozoa in the rat.

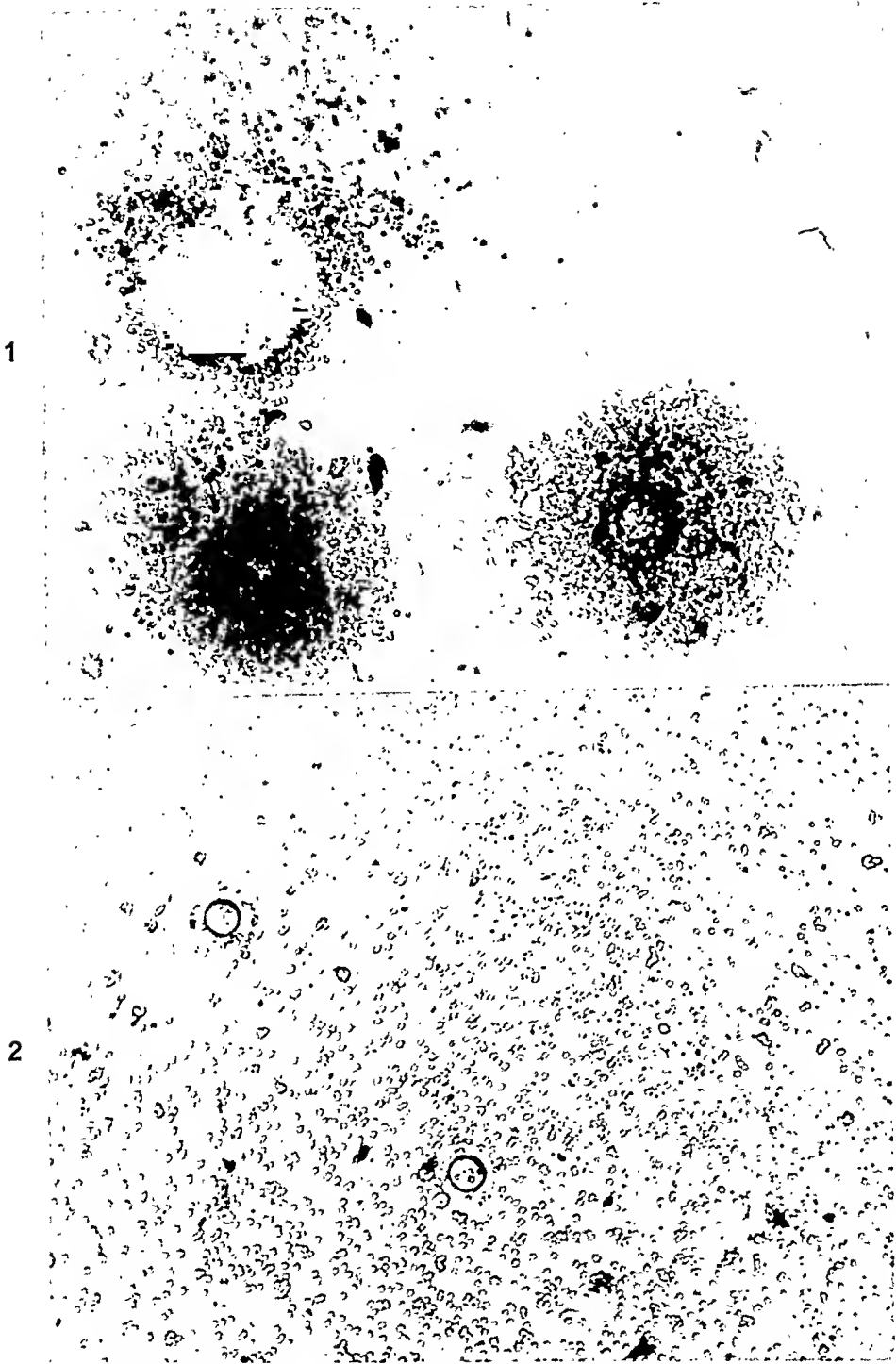


FIG. 1. Ovulated rat ova in Ringer's solution for four hours. The granulosa cells are closely adherent to the ovum. Relatively few granulosa cells are seen free in the Ringer's solution.

FIG. 2. Ovulated rat ova in Ringer's solution *plus* hyaluronidase after four hours. The granulosa cells have been dispersed and fill the surrounding fluid. The ova are almost completely denuded, except for a single layer of granulosa cells.

Previously reported studies² showed the following:

1. A simple test for hyaluronidase in human semen was described, based on the formation of a ring when hyaluronic acid is added to acidified blood serum (usually human). In the presence of hyaluronidase a ring will not form. Clear seminal plasma, obtained by means of the Boerner centrifuge filter, is essential.

2. The hyaluronidase content of human semen, as studied in more than 400 specimens, increases directly with the sperm concentration up to the range of 100 million sperms per cc. Beyond this concentration the hyaluronidase content does not increase proportionately. Semen specimens containing less than 50 million sperms per cc. rarely contain the enzyme.

3. Semen specimens with excellent concentrations of sperm may not contain any hyaluronidase. We consider such specimens infertile.

4. The hyaluronidase content of semen does not appear to be related to the morphology, motility or "percentage" of motility of sperms.

5. On the basis of the above findings, hyaluronidase was utilized clinically in the treatment of sterility. The enzyme was added directly to the semen which was then used for artificial insemination; or from 10 to 20 mg. of the enzyme was packed into the cervix at the time of suspected ovulation, or on the day prior to it, and the patient was instructed to have coitus. To the 6 successful cases originally reported, we now can add 33 additional successful cases out of a total of 102 patients chosen as suitable for hyaluronidase therapy.

The patients chosen for hyaluronidase therapy were subjected to the usual complete routine study. This included complete physical examination of both husband and wife, tubal insufflation, endometrial biopsy, determination of the approximate date of ovulation by the vaginal smear method of Papanicolaou and Shorr,¹⁰ basal metabolism, blood count and whatever additional tests may have been required. A complete study of the semen was made, which included a semen-mucus penetration test³ and a quantitative estimation of hyaluronidase by the ring method previously described.

Hyaluronidase does not liquefy cervical mucus, a fact previously noted by Rowlands;¹² hence, the enzyme is not identical with the one present in semen which was described by Kurzrok and Miller.³ A certain parallelism, however, does appear in the two enzymes. Good penetration of the cervical mucus by sperm requires a sperm population of 40 million per cc. or more. This seems to be the level below which hyaluronidase usually cannot be demonstrated. On the other hand, sperm concentrations of 100 million per cc. or more, showing no hyaluronidase, may exhibit excellent penetration of cervical mucus. Apparently, a minimum population of sperms is necessary for effective action of enzymes.

Table 1 presents the findings in a series of 33 women who conceived after hyaluronidase therapy. Fifteen patients conceived after one or two treatments; that is, conception occurred during the first or second month after the enzyme was applied. Patients were seen only once during the cycle, at the time of ovulation. Several patients (Table 1) presented special problems.

Patient No. 20 presented no abnormality. The hyaluronidase content was normal. We applied hyaluronidase during four successive months and the patient conceived during the month after the fourth treatment. Our therapy was

TABLE 1

DETAILED DATA OF 33 PATIENTS IN WHOM THE THERAPEUTIC USE OF HYALURONIDASE RESULTED IN PREGNANCY. A TOTAL OF 102 PATIENTS WAS CHOSEN AS SUITABLE FOR HYALURONIDASE THERAPY; THERAPY FAILED IN APPROXIMATELY 70 PER CENT OF THE PATIENTS

PATIENT NUMBER	SPERM COUNT	HYALURONIDASE TITER	ABNORMAL MORPHOLOGY	TREATMENTS WITH HYALURONIDASE	DURATION OF STERILITY	REMARKS
	millions per cc.	units per cc.	per cent	number	years	
1	68	0	30	1	2	
2	22	0	45	1	3	
3	92	0.5	25	2	5	
4	28	0	60	2	1½	
5	70	0.5	40	2	1	Spontaneous miscarriage at 7 weeks.
5A	74	0.5	45	2		
6	98	0.5	28		2½	Between third and fourth application of hyaluronidase, patient had a Trichomonas infection which was treated.
7	Record lost			1	4	
8	73	0.5	40	3	6	
9	146	0.5	20	1	3	Miscarried at 7 weeks.
9A	—			1	3½	
10	35	0	70	4	2½	
11	15	0.5	30	5	2	
12	48	0.5	40	4	2½	
13	80	0.5	40	4	2	
14	105	1.0	35	4	3	Hyaluronidase of dubious value as it was combined with papain for purulent discharge.
15	140	1.0	25	2	2	
16	80	0.5	30	6	2½	
17	192	1.0	30	7	2	
18	167	0.5	30	1	4	
19	—	1.0	30	6	1	
20	210	1.0	30	4	1	
21	60	0	28	2	1	
22	100	0.5	12	2	1	
23	89	0.5	40	6	4	
24	20	0.5	50	3	1	
25	136	Dubious	20	2	1	
26	122	1.0	20	9	2	Patient had endocervicitis; on ninth attempt 5 mg. papain was added.
27	Record lost			6	2	Findings lost.
28	100	0.5	20	2	2	
29	180	1.0	30	3	4	
30	110	1.0	—	3	5	
31	155	1.0	15	4	1	
32	51	0	65	4	1	
33	60	0.5	20	5	2	

based on the concept that the concentration of enzyme required to depolymerize the hyaluronate in the cumulus of the ovary varies in different individuals.

Patient No. 14 presented no abnormality except a nonspecific mucopurulent cervical discharge. We had previously noted that sperm penetration did not take place through infected or very gelatinous mucus. It is our custom, in patients with endocervicitis, to pack the cervical canal with hyaluronidase and 5 mg. of papain on the day of suspected ovulation. Papain liquefies cervical mucus. The usual amount of enzyme for such action is about 5 mg., but a larger amount may be necessary in some cases. Greater concentrations of papain cause rapid immobilization of sperm due to possible digestion of the proteins in the seminal plasma. In view of the fact that the hyaluronidase concentration was greater than 1.0 unit, its efficacy in this case must remain dubious.

Patient No. 26 presented a problem somewhat similar to that of patient No. 14. Endocervicitis was present, but was not considered significant. Eight successive months of therapy with hyaluronidase failed to lead to pregnancy. The addition of 5 mg. of papain during the ninth month, however, was followed by success.

Whenever a seminal specimen was considered inadequate because of morphology, low count, or other reason, a second specimen was obtained, usually within two weeks, after sexual abstinence of one or two weeks. Table 2 presents a group of 22 consecutive cases. These cases indicate that: (1) Sexual abstinence does not increase the hyaluronidase content of semen. (2) If hyaluronidase is absent in the first specimen, it is usually absent in the second, unless there is a marked increase in the sperm count.

Our present method of application of hyaluronidase is as follows. The patient is seen on the expected day of, or on the day prior to, ovulation. The cervix is exposed by a speculum and the excess mucus over the external os is wiped off. No germicidal agent is used. A thin sterile cotton applicator is then gently inserted into the cervical mucus of the external os. The applicator should not be pushed into a narrow cervical canal lest bleeding occur, since we consider active bleeding to be detrimental. The applicator will then have adherent to it minute strands of cervical mucus. Ten to 20 mg. of hyaluronidase, depending on the available supply, is placed on a watch glass. The applicator is rolled in the enzyme on the watch glass and is then reinserted into the external os. By gently rotating the applicator the adherent enzyme will remain in the mucus of the cervical canal. The entire maneuver may be repeated until all the enzyme on the watch glass has been transferred into the cervix. Dipping the clean applicator into the cervical mucus as the first step has the advantage of making the enzyme adhere to the cotton. The highly purified enzyme is very fluffy and will not adhere in sufficient quantity to dry cotton. The patient is advised to have coitus as soon thereafter as possible.

We do not consider this method of hyaluronidase application entirely adequate for several reasons: (1) The date of ovulation varies, and it is not feasible to determine it every month; hence, the day most suitable for application of enzyme may be missed. (2) The patient cannot always be seen by the physician on the date of ovulation. (3) Coitus may have to be postponed more than twelve hours after the application of the enzyme.

TABLE 2

DETAILED STUDY OF HYALURONIDASE VALUE OF SEMEN SPECIMENS COLLECTED FROM THE SAME INDIVIDUALS AT INTERVALS ONE OR TWO WEEKS OR LONGER, A SEXUAL REST INTERVENING; 5 PLUS DENOTES MAXIMAL MOTILITY, 0 DENOTES ABSENCE OF MOTILITY

PATIENT	DATE	AGE	VOLUME	MOTILITY		AB-NORMAL	SPERM COUNT	VISCOSITY	HYALURONIDASE
number		hours	cc.	per cent*	degree	per cent	millions		units
34	1/29/47	2	3		3-4	—	0	normal	0
	3/ 3/47	1½	6		0	—	0	watery	<0.25
35	6/20/46	1½	3½	70	5+	15	54	normal	0
	1/29/47	2	2½	50	4+	30	50	normal	<0.5
36	11/26/47	3	1	80	5+	10	170	normal	>1.0
	12/24/47	1½	1	70	5+	20	109	normal	<1.0
37	11/25/47	1¼	3	70	5+	10	325	normal	<1.0
	12/ 4/47	2½	3	70	5+	10	140	normal	<1.0
38	3/26/47	1½	1.5	10	4+	40	32	viscous	<0.3
	4/ 7/47	2½	4	50	5+	40	80	normal	0.5
39	3/26/47	2	2.5	0		50	—	normal	<0.5
	4/ 9/47	2	3	50	5+	40	45	normal	0
40	4/11/47	3	1	5	4+	60	3-4	viscous	<0.5
	4/28/47	½	2.5	0		90	5-6	slightly viscous	0
41	12/19/47	3	1.5	20	3+	40	40	normal	>0.5
	4/16/47	2½	2	10	4+	70	24	slightly viscous	—
42	1/20/47	2½	3	—		—	1-2	watery	<0.3
	4/21/47	4	3	0		—	2-3	watery	—
43	5/ 7/47	2	0.5	0		90	2-3	normal	0
	5/26/47	2½	3	0		80	10	watery	0
44	2/20/47	1	2.5	70	5+	50	90	normal	—
	6/19/47	½	1	50	5+	40	80	slightly viscous	<0.5
45	5/12/47	3	0.8	10	4+	80	3-4	normal	<0.5
	5/26/47	1	2	50	4+	80	1-2	normal	0
46	5/ 9/47	2	1	40	5+	50	70	normal	<0.5
	6/30/47	2½	1	60	5+	60	16	normal	<0.5
47	5/16/47	2½	1.5	40	4+	50	4-5	slightly viscous	0
	5/28/47	2	5	0		60	20	normal	<0.5
48	5/16/47	2½	2	10	1+	60	30	normal	<0.5
	5/29/47	3	2	10	1+	40	170	normal	<1.0
49	1/26/47	2	2.5	60	5+	40	170	normal	0.5
	5/20/47	2	2	60	5+	40	79	very viscous	0
50	5/21/47	½	3	50	5+	60	24	watery	—
	6/18/47	2½	3	50	5+	15	83	watery	<0.5
51	5/22/47	8	2	0		80	10	normal	0
	6/ 3/47	2	3	40	5+	60	125	normal	<0.5
52	5/28/47	3	1	10	3+	85	15	normal	0
	6/12/47	1	1	50	5+	50	20	normal	0
53	6/10/47	2	2	10	5+	85	44	normal	0
	6/19/47	2	2	20	5+	70	89	normal	>0.1
54	6/17/47	2	2.5	75	5+	15	143	normal	<1.0
	6/26/47	2	1	70	5+	20	80	normal	—
55	6/ 2/47	2	2	30	5+	80	60	normal	0
	6/18/47	2½	3	70	5+	40	88	watery	<1.0

* Per cent of sperms showing motility.

It would be of greater advantage if the patient herself could insert a preparation containing the enzyme daily during the entire fertile period. Coitus could then follow immediately after the insertion of material containing the enzyme. Studies with this method are now in progress.

SUMMARY

1. Hyaluronidase was found to be of therapeutic value in human infertility in 33 of 102 selected patients. The application of the enzyme may be of value in the following instances: (a) In patients presenting a sperm concentration of less than 50 million per cc., because the enzyme is usually absent when the sperm population is below this level. (b) In patients presenting a normal sperm concentration (about 100 million sperms per cc.), where the enzyme concentration is low, that is, less than 1 unit. (c) Occasionally in a patient with a normal sperm population and adequate hyaluronidase (1 or more units per cc.), in whom no adequate reason can be found for the infertility.

2. Hyaluronidase and the enzyme that liquefies cervical mucus are not identical but present certain parallelisms.

3. The addition of the enzyme papain to hyaluronidase may be of distinct value in patients with a nonspecific endocervicitis.

4. Sexual abstinence for a limited period (average about two weeks) does not increase the hyaluronidase content of semen.

Acknowledgment. I wish to thank Professor Samuel Leonard of Cornell University for conducting the non-clinical investigations on hyaluronidase. We are indebted to Dr. E. Schwenk and Dr. E. Henderson of the Schering Corporation, Bloomfield, New Jersey, for the supply of hyaluronidase and hyaluronic acid. The author also wishes to express his appreciation to Miss Irene Paulin and Mrs. Barbara Saur for the enzyme analysis reported in this paper.

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AGGLUTINATION RESPONSE FOLLOWING INTRACUTANEOUS SKIN TESTING WITH *BRUCELLA ABORTUS* ANTIGEN*

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Since 1929 when Giordano and Ableson⁵ introduced the skin test in the United States for the diagnosis of undulant fever, wide variations have been reported in the maximum titers obtained in agglutination reactions following intracutaneous testing. Giordano⁴ was among the first to note that an antibody response could occur, for he observed that appreciable agglutinin titers were induced within a period of a few days to three months, not only in those individuals who reacted positively to the skin test, but also in 70 per cent of his control group who had negative skin tests. His dosage was 0.2 cc. of heat killed bacterial suspension of *Brucella abortus*, corresponding in concentration to the 1:1000 silica standard of the United States Public Health Service.

Specific determinations of the agglutinin titers obtained after skin testing with heat-killed suspensions and other types of antigens have been reported.^{1, 2, 6-8, 11, 13} The findings of these investigators have differed considerably, ranging from titers as high as 1:10,240 reported by Goldstein,⁶ to insignificant responses reported by others.^{8, 11, 13} The actual dosages employed by various workers are not comparable, since some used heat-killed suspensions, some used fat-free antigen and some used the protein derivative (brucellergen), usually in different amounts and in various concentrations. Some of the dosages of heat-killed organisms, however, can be estimated accurately. Goldstein injected 0.05 cc. of a saline suspension of *B. abortus* number 80, containing 500 million organisms per cc., which may be computed as a measurable skin test dose of 25 million organisms. Kirby and Rantz¹¹ used a skin dose of 40 million organisms and Elton¹ used a skin dose of 200 million organisms. One would expect, then, that the agglutinin response found by Goldstein would be somewhat less than that found by Kirby and Rantz, yet the reverse was true, for the latter concluded that, except when fever was present, the response was so low (no reaction in 50 per cent and no titer above 1:80), that it need cause no confusion in the interpretation of agglutination reactions developing after a skin test had been performed. The high dosage employed by Elton might be expected, on the other hand, to act as an effective vaccinating dose with the occurrence of a titer up to 1:1280. In the same way, Tuft¹⁸ found that a high titer of typhoid agglutinins developed after a corresponding dose of *Eberthella typhosa* was similarly employed. Since Goldstein employed a smaller skin dose, but reported a far higher titer of agglutinins than did Kirby and Rantz, there must have been an appreciable difference in the antigenic potency of the bacterial suspensions used.

* Read by title at the Twenty-Sixth Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 29, 1947. Received for publication, October 27, 1947.

A review of the literature supports the following statements:

- a. The antibody response following the skin test is not related to the positivity or negativity of the skin reaction, since reactions frequently occur following negative skin tests.^{1, 4, 6}
- b. Although many individuals exhibit an agglutinin response following skin tests, some do not develop any at all.

TABLE 1
ABORTIN SKIN TEST WITH DOSE OF 200 MILLION HEAT-KILLED ORGANISMS
DATE OF TEST, MARCH 13, 1940

SUBJECT	SIZE AND INTENSITY OF REACTION IN INDICATED NUMBER OF HOURS FOLLOWING TEST		
	24 Hr.	72 Hr.	144 Hr.
W. S.	7 x 5 cm. 3+	7 x 5 cm. 2+	1 cm. 1+
M. W.	7 x 5 cm. 3+	2 x 2 cm. 2+	2 x 2 cm. 1+
E. S.	5 x 4 cm. 3+	0	0
H. W.	5 x 4 cm. 3+	spot 1+	0
R. F.	1+	0	0
H. H.	0	0	0
E. A.	5 x 4 cm. 3+	0	0
N. E.	14 x 8 cm. 4+	14 x 8 cm. 4+	Necrosis

Positive reactions are based on combination of redness, edema and central induration; degree of reaction on diameter of reaction as follows: 4+, over 6 cm.; 3+, 4 to 6 cm.; 2+, 2 to 4 cm.; 1+, 1 to 2 cm.

- c. The agglutinin titer prior to the performance of a skin test is not relevant to the degree of response following the skin test in individuals not ill with active undulant fever.⁶
- d. Antibody responses following skin testing will vary with different antigens, with different test subjects and with different skin dosages.

EXPERIMENT

The following experiment is presented to help clarify this somewhat controversial subject.

In 1940, a neurotic, elderly patient with periodic pyrexia of undetermined etiology and of one year's duration was found to have no agglutinins for *B. abortus* in tests performed by

three clinical laboratories. Not satisfied with these findings, his physician performed an endermal test with a killed suspension of *B. abortus* which yielded a positive reaction, and subsequently submitted a specimen of serum to a fourth laboratory out-of-town, where agglutination to a titer of 1:1280 was promptly reported. On this basis a diagnosis of

TABLE 2

AGGLUTINATION TITERS USING THREE ANTIGENS. VALUES OBTAINED BY FOURTH REFERENCE LABORATORY THREE WEEKS AFTER SKIN TESTS (200 MILLION ORGANISMS)

TEST SUBJECT	ANTIGENS*	GRADE OF REACTIONS IN DILUTIONS INDICATED					
		1:10	1:20	1:40	1:80	1:160	1:320
W. S.	1	4	4	4	4	4	4
	2	4	4	4	4	4	4
	3	0	2	3	3	3	3
M. W.	1	4	4	4	4	4	4
	2	4	4	4	4	4	4
	3	3-2	3	4-3	4-3	4-3	3-2
E. S.	1	4	4	4	4	4	4-3
	2	4	4	4	4	4	4
	3	4	4	4	4-3	4-3	3-2
H. W.	1	4	4	4	4	4	4-3
	2	4	4	4	4	4	4
	3	4	4	4	4-3	4-3	3-2
R. F.	1	0	0	0	0	0	0
	2	0	0	3	4	3	0
	3	0	0	2	2	0	0
H. H.	1	4-3	4-3	4	4	4	3-2
	2	4	4	4	4	4	4-3
	3	4	4	4	4-3	3	1
E. A.	1	4	4	4	4	4	4-3
	2	4	4	4	4	4	4
	3	4	4	4	4	4-3	2
N. E.	1	0	4	4	2	0	0
	2	4	4	3-2	0	0	0
	3	0	0	2	2	0	0

* Antigens: 1, killed suspension *B. abortus*; 2, living suspension *B. abortus*; 3, *B. melitensis*.

undulant fever was made. The original tests were considered erroneous and misleading, and the three laboratories originally involved were accused of unreliability.

Believing that it might be fallacious to make a diagnosis of active undulant fever on the basis of a skin test alone and particularly to make a diagnosis on the basis of an agglutination test following a skin test in which a suspension of bac-

terial bodies was employed, an investigation was undertaken to determine whether or not the skin test itself could have provoked the antibody response.

Seeking to check the validity of this diagnostic device, 8 workers in the laboratory where the initial agglutination test was reported as negative, were given skin tests with Hauenstein's bacillary body suspension prepared for intracutaneous use. The dosage was 0.1 cc. of a suspension of 2000 million organisms (*B. abortus*) per cc. (barium sulfate number 3 standard). None of the group had ever had a clinically recognizable attack of an illness that resembled undulant fever at any time of life. All but one (R.F.) had drunk raw milk during childhood. All were actively working adults in excellent health at the time the tests were conducted, and none were in any way incapacitated by the tests. The results of these skin tests are presented in Table 1.

One week after the skin tests the serums of 2 of the test subjects (M.W. and N.E.) were tested for agglutinins and none were found. The omission of this

TABLE 3
AGGLUTINATION TITERS OBTAINED BY FIFTH REFERENCE LABORATORY THREE WEEKS
AFTER SKIN TESTS (200 MILLION ORGANISMS)

TEST SUBJECT	TITER
W. S.	1:160
M. W.	1:320
E. S.	1:320
H. W.	1:80
R. F.	"Partial"
H. H.	1:160
E. A.	1:160
N. E.	"Partial"

determination at this time on the other 6 appears of little importance in consideration of the subsequent developments.

Three weeks after the skin tests, blood was taken from each of the 8 subjects, and the serums of all but 2 were found to agglutinate a killed suspension of *B. abortus* in a titer of 1:1280. The serum from the subject with the severest skin reaction (N.E.) and from another subject with a very weak reaction (R.F.) agglutinated unequivocally but only up to a titer of 1:640. Samples of these same serums were sent to the fourth out-of-town laboratory previously mentioned and also to a fifth city laboratory. The reports of these two reference laboratories are presented in Tables 2 and 3, respectively. Neither of these laboratories had set up the test for titers above 1:320. Prozone reactions were reported only by the fourth laboratory, primarily when *Brucella melitensis* was used as the antigen or in the 1:10 and 1:20 dilutions of *B. abortus*. They could have been overlooked by the original workers.

Five weeks after the skin tests the agglutinin titers were again determined by the original group of workers, with the results shown in Table 4. At this stage a slight recession is noted.

DISCUSSION

This experiment conclusively demonstrated that a specific antibody response followed the intracutaneous inoculation of 0.1 cc. of a suspension of *B. abortus* in a concentration of 2000 million organisms per cc. (a skin dose of 200 million organisms) and that this response was fully comparable to that which might be expected following vaccination by the subcutaneous or intramuscular technic using materially higher dosages. It is also obvious that the degree of skin sensitivity bore no demonstrable relationship to the intensity of the subsequent antibody response.

The antibody response induced by this type of skin test was consistent with the findings of Siler and his co-workers,¹⁵ and by Tuft,¹⁸ who worked with the typhoid bacillus. Re-immunization with typhoid vaccine by the intracutaneous technic was extensively studied by Siler, who concluded that the single booster dose could be 0.1 cc. administered intracutaneously, or 0.5 cc. administered subcutaneously,

TABLE 4

AGGLUTININ TITERS WITH *B. abortus* ANTIGEN VALUES OBTAINED BY ORIGINAL LABORATORY FIVE WEEKS AFTER SKIN TEST (200 MILLION ORGANISMS)

TEST SUBJECT	GRADE OF REACTION IN DILUTIONS						
	1:20	1:40	1:80	1:160	1:320	1:640	1:1280
W. S.	4	4	3	2	1	0	0
M. W.	3	3	2	1	±	0	0
E. S.	4	3	3	2	2	1	1
H. W.	4	3	2	1	1	1	±
R. F.	3	2	1	±	±	0	0
H. H.	4	3	2	1	±	0	0
E. A.	4	3	2	1	1	0	0
N. E.	3	3	2	±	0	0	0

the former being the method of choice. Tuft found that the intracutaneous technic for the initial vaccination, in doses of 0.1 cc., 0.15 cc. and 0.2 cc. at weekly intervals gave slightly higher agglutinin titers for the H-antigen than did the standard subcutaneous technic, with equally satisfactory titers for the O-antigen in individuals never previously immunized, and that mouse protection tests were better with the intracutaneous group than with the subcutaneous group or with convalescent serum. Single dose booster inoculations into the skin were also found more effective than larger single inoculations subcutaneously or intramuscularly in the mouse protection tests. Tuft,¹⁶ as early as 1931, definitely recommended the intracutaneous technic for routine use in typhoid vaccinations, because of the milder degree of local reaction and the absence of constitutional symptoms, as well as the obvious advantage of attaining the desired result with approximately one-tenth of the conventional dose. The typhoid suspension used by Tuft was 2000 million organisms per cc.

Pasteur originally recognized that the skin is an important immunologic organ, participating very actively in antibody production, and this property has been

discussed by Tuft.¹⁷ In this connection, Plotz,¹⁴ a Director of the Pasteur Institute in Paris prior to World War II, called attention to the original work of Pasteur with *Bacillus anthracis*. A little known phase of Pasteur's original work with the anthrax bacillus is that he was fully cognizant of the great disparity that exists between conventional antibodies and effective protective substances. He had noted that subcutaneous inoculations of killed anthrax suspensions provoked excellent antibody responses, but afforded no protection whatever against subsequent anthrax infections, thus clearly demonstrating the difference between antibodies, such as agglutinins, and true protective substances. Pasteur did discover, however, that he could produce protection in animals by intracutaneous inoculations of attenuated strains of living anthrax bacilli after preliminary intracutaneous inoculations of suspensions of killed organisms. This apparently poorly known aspect of Pasteur's researches indicates the specific role of organ immunity, such as the skin, and perhaps many mucous membranes, as the initial barrier to invasion by pathogenic agents.

The possibilities implied in the intracutaneous technic for immunization have been explored in diphtheria⁹ and in scarlet fever.¹⁰

The status of the skin test for undulant fever has now come to be regarded as quite analogous to that of the tuberculin test, in that it establishes only that brucellosis has existed at some time in the past and affords little help in indicating the presence of active disease.

When Giordano and Ableson reported the early results of the skin test in 1929, evaluation was difficult, for it was immediately recognized that the problem of the standard test dose remained to be solved. They initially used 0.12 cc. of *B. abortus* vaccine with a turbidity corresponding to the 1:500 U. S. P. H. S. silica standard, and then reduced the dosage to 0.05 cc. of the 1:1000 standard with equally good results. Subsequently, work has been conducted with the nucleoprotein, brucellergen, in different dilutions. Evans² has reported titers as high as 1:320 following the administration of this type of antigen, although Huddleson states that "One skin injection of brucellergen may give rise to brucella agglutinins in a low titer in a small percentage of individuals".

Although there has been a progressive diminution in the skin dosage in recent years, and that now recommended by the Massachusetts Department of Public Health is only 8 million organisms in suspension,¹² this agency still advises deferring the skin test until all information obtainable from the agglutination test has been secured.

The problem of the skin test is largely a diagnostic one, but it seems that the dosage of nucleoprotein or bacterial suspension used in skin testing still remains controversial in determining the concentration that is just right in all individuals to indicate either previous infection or active disease, and the dosage of a specific antigen which will provoke a minimal subsequent antibody response.

If, on the other hand, vaccination against brucellosis is contemplated, the endermal technic of administration is recommended, using dosages of at least 200 million heat-killed organisms obtained from a culture of *B. abortus* of high antigenic potency.

SUMMARY

The intracutaneous test for brucellosis may be followed by the appearance in the serum of agglutinins for *Brucella abortus*, the titer of which is not dependent on the result of the skin test, but is probably chiefly dependent on the amount and type of the antigen used (antigenic potency) for skin testing.

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VARIATIONS IN BRUCELLA AGGLUTINATION REACTIONS IN DIFFERENT LABORATORIES*

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The present study reports data showing variations in the Brucella agglutination reaction when the same bloods were tested simultaneously in 4 different laboratories. In preparation of the antigens for the test, only 2 of the 4 laboratories used subcultures of the same strain of *Brucella abortus*. Agglutination tests were performed on blood from 215 patients simultaneously in 2 or more of the 4 laboratories. Thus, each blood specimen was tested with from 2 to 4 different antigens of *B. abortus*.

Sixty-four, or 30 per cent, of these tests showed results which were negative in one laboratory and significantly positive in another. Only 19 tests were positive in all laboratories. Uniformly negative reports were received from all laboratories in 132 patients (60 per cent). Of these latter patients more than one-half were presumed to have no brucellosis after all other diagnostic studies were completed.

In Table 1 are listed the results of tests in 24 of the patients. The table indicates that some laboratories used relatively insensitive antigens of *B. abortus*, and yet there are a few instances (patients 8, 9 and 24) in which these strains may have detected the disease more readily than antigens which were generally more sensitive. The least sensitive antigen in this study was prepared from N. I. H. strain #456, a widely used and approved strain. Laboratories number 2 and 3 used strain #80, isolated by Dr. Karl Meyer of San Francisco. Laboratory number 4 (Pomona Valley Hospital) used an antigen prepared from a mixture of 5 strains selected for consistency of results and sensitivity following a study by Angle, Algie and Morgan¹ in 1942. This antigen gave more positive reactions than any of the others, but it was not always more sensitive. It did not give false positives, as far as we could tell, in titers of 1:40 and higher. It is obvious from Table 1 that there is a wide divergence of results in Brucella agglutination tests in different laboratories.

Whether an agglutination reaction is of significance may depend not merely on its titer but also on the evaluation of other findings in the case, *viz.*, the agglutination reactions for other diseases which may give cross-reactions, the opsonocytophagic and complement-fixation reactions, and whether the patient has received Brucella vaccine or recent skin tests with Brucella substances. For this reason the laboratory should refrain from giving interpretations of the reactions, but should report only what is found objectively. Phrases such as "too low for diagnosis" or "not diagnostic in this titer" should be avoided.

Griffitts,² in an important work, has recently shown that serums from persons

* Received for publication, March 22, 1948.

TABLE 1

VARIATIONS IN AGGLUTINATING TESTS* FOR BRUCELLOSIS IN 24 PATIENTS. AGGLUTINATION TESTS WERE PERFORMED SIMULTANEOUSLY IN FOUR DIFFERENT LABORATORIES. COMPLEMENT-FIXATION AND OPSONOCYTOPHAGIC TESTS WERE PERFORMED IN LABORATORIES NO. 3 AND NO. 4

PATIENT NO.	RESULTS OF AGGLUTINATION TESTS IN LABORATORY				OTHER TESTS	
	No. 1	No. 2	No. 3	No. 4	Complement Fixation	Phagocytic Index No.
1	neg.	neg.	1:80++++	1:50	neg.	35
2	neg.	neg.	1:40	1:80	1:4++++	92
3	neg.	neg.	1:640++++	1:400	1:64++++	90
4	neg.	neg.	1:80++++ 1:160++++	1:200	1:64++++	86
5	neg.	neg.	1:160++++ 1:320++++	1:80	1:8++++	82
6	neg.	neg.	1:320++++ 1:640+	1:100	1:64++++ 1:128++ 1:256+	87
7	neg.	neg.	1:160++++	1:200	1:16++++ 1:32+++	70
8	1:80	neg.	1:320++++	1:200	1:16++++	
9	1:80		neg.	1:200	neg.	83
10		neg.	1:160++++	1:200	1:32++++	33
11		neg.		1:1280		96
12		neg.		1:100	pos.	69
13	neg.	neg.	neg.	neg.	1:16++	63
14		neg.	neg.	1:50	1:64++++	73
15	neg.	1:100	1:640+++	1:200		42
16	neg.	1:100	1:640	1:200	1:32++++	70
17	neg.	1:100	1:16++++	1:160	1:32++++ 1:64+++	92
18	neg.	1:100 +-1:200	1:640++++ 1:1280+++	1:400	1:128++++ 1:256+++	
19	neg.	1:200	1:640	1:160	1:16++++	32
20	neg.	1:100	1:640++++	1:100	1:256++++	46
21	neg.	+-1:200		1:400		68
22	neg.		1:80++++	1:100	1:8++	31
23	neg.		1:160++++	1:200	1:2+	44
24	neg.	1:50	1:160++++	neg.	neg.	70

* Laboratory No. 1 used an antigen prepared from N. I. H. strain #456, laboratories No. 2 and No. 3 used antigens prepared from strain #80 and laboratory No. 4 used an antigen prepared from a mixture of 5 strains selected for consistency of results and sensitivity from among 20 strains.

known to have been infected with *Brucella* have the same property of "blocking" the agglutination as is found in Rh sensitization. From his study it is clear that all *Brucella* agglutination tests should be done with serum in place of saline as a diluent for titrations and as a suspending medium for *Brucella* antigens.

Table 1 includes the results of the Brucella complement-fixation and opsonocytophagic reactions as evidence that the positive agglutinations in these cases are not false positive. When the agglutination reaction is falsely positive, it is usually due to the presence of some other infection which can give a cross-agglutination, or it may be obtained after Brucella substances have been injected in skin testing, or given therapeutically or after a recent vaccination against cholera. Agglutinins may or may not persist after complete recovery from acute undulant fever.

The Brucella complement-fixation reaction is sometimes positive when the agglutination test is negative, and *vice versa*. The antigen used in the complement-fixation tests in this study was made from strain #80, the same strain that was used for the agglutination antigen in laboratory number 3; yet, it is seen in Table 1 that the two reactions do not always support each other even in the same laboratory. By itself, the complement-fixation test is no more valuable than the agglutination reaction alone. Both should be used with all other means of diagnosis of the disease.

The opsonocytophagic index is considered to be of more value in brucellosis than it is in tuberculosis and other infections in which it has been used. Yet, it is difficult to interpret in many cases. While a very high phagocytic index number (Foshay), 80 to 100, suggests immunity, we now know that this immunity may be limited to the blood stream or the blood cells. The very low index number, 0 to 5 or 10, may be interpreted either as "probably no infection", or as "no opsonic response", depending on the other findings in the case.

After vaccine therapy, accurate diagnosis may be impossible. After skin tests, false positive serologic reactions may appear even in normal, uninfected persons.

SUMMARY

Significant variations in the Brucella agglutination reaction were reported in different laboratories on the same blood specimens, presumably because of the use of different antigens.

It is believed that some antigens are so insensitive that they tend to produce false negative reactions and that diagnostic errors are consequently frequent.

In the agglutination test the use of antigens made from multiple strains of *B. abortus* is recommended.

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ISOLATION OF SHIGELLA FROM THE GALLBLADDER IN BACILLARY DYSENTERY*

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Recently, A. J. Levy² reported the isolation of *Shigella ambigua* from the gallbladder and of *Shigella sonnei* from the ascending colon at autopsy of a girl who was an epileptic and idiot. The patient had suffered more than six years from intermittent diarrhea; but, although 329 cultures were taken by the swab method during the last year of her life, *S. ambigua* had not been found prior to autopsy. From these 329 cultures, 110 were positive for *S. sonnei* and 4 were positive for *Shigella paradysenteriae*. Therefore, the infection with *S. ambigua* must have been contracted shortly before death, or this organism must have been hidden in the gallbladder since the beginning of her illness and before bacteriologic examinations were performed.

The isolation of *Shigella* from the gallbladder, however, is not as rare as may be supposed. Lentz and Prigge¹ cited several instances in the *Handbuch der pathogenen Mikroorganismen*. One of these instances which may be well compared with Levy's case is that of Tscherning (1922). The latter isolated *S. paradysenteriae* from the gallbladder removed by surgery from a patient who had suffered from bacillary dysentery four years previously.

Since September 1944, we conducted bacteriologic examinations for *Shigella* infection of the gallbladders of all subjects who came to autopsy with a diagnosis of bacillary dysentery. Sometimes this diagnosis was only tentative, as when a moribund patient was hospitalized and died before a complete clinical examination could be made. The first postmortem culture of the gallbladder was made because this viscus appeared to be abnormally distended. This culture was positive. At subsequent autopsies, the gallbladder with the surrounding liver tissue and a part of the colon were removed from the body and sent to the Bacteriologic Department of the Public Health Laboratory. Between September 1944 and December 1947 *Shigella* was isolated 11 times postmortem. All patients were young children whose ages varied from 3 to 17 months. Eight times the culture from the colon was positive and that from the gallbladder negative. Three of these positive cultures were *S. sonnei*, and five belonged to the Flexner group (*S. paradysenteriae*). In three instances in which *S. paradysenteriae* was isolated from the gallbladder, simultaneous cultures of the colon were negative.

REPORT OF CASES

Case 1

A Negro girl of 17 months was hospitalized on September 8, 1944. The child was moribund and died three hours later. She had been ill at home for one week with diarrhea and fever. The mother had treated the child with a diet of biscuits and tea. At the onset

* Received for publication, January 9, 1948.

of the illness, the stools had a normal color; later the stools became spinach green, and blood and mucus were passed. As the general condition deteriorated, a physician was consulted and the child was referred to the hospital.

The patient presented a typical picture of dehydration with hollow abdomen and wrinkled skin. The pulse was not palpable, and the heart sounds were soft and rapid. The liver was enlarged, its inferior margin being palpable three fingerbreadths beneath the costal arch. The temperature was 101.3 F., the leukocyte count, 12,600 per cu. mm. Microscopic examination of feces revealed numerous leukocytes but no amebae or ova. Treatment consisted in infusion of Ringer's solution in the external jugular vein and in cardiotonics. Autopsy was performed (by v. d. S.) fifteen minutes after death. The liver was enlarged and yellow, and the gallbladder was large and contained a great quantity of bile. Peyer's plaques in the ileum were slightly swollen, and the mucous membrane of the descending part of the colon was red and swollen.

Bacteriologic and serologic examinations of feces and blood were negative for typhoid, paratyphoid and dysentery. No pathogens were isolated from the colon. A culture on SS-agar from the mucous membrane of the gallbladder revealed the presence of *S. paradysenteriae*.

Case 2

A Negro girl, aged 12 months, was hospitalized on January 2, 1945 with a diagnosis of "acute enterocolitis". The patient's history and present condition showed a striking resemblance to those of Case 1. The child had been ill for five days with fever, diarrhea and abdominal pains. The stools contained mucus and were greenish tinged, but blood had never been seen. Medical aid was consulted, and a diet and astringents were prescribed. However, the symptoms did not abate in severity.

At the time of hospitalization the child was emaciated and presented a wrinkled skin and a hollow abdomen. The spleen was not palpable, and the liver was enlarged. Microscopic examination of the stools revealed many white blood cells but no amebae or ova. Infusion of isotonic salt solution in the external jugular vein and hypodermoclysis were given, followed by a transfusion of blood from the mother, and 275 mg. sulfaguanidin was given every three hours *per os*. The patient died the next day.

Autopsy was performed (by P. H. H.) two hours after death. Grossly, there was fatty infiltration of the liver, and the mucosa of the large intestine was hyperemic and slightly swollen. Microscopic sections of the colon revealed some superficial erosions of the mucous membrane, especially in places where the submucosa contained lymphoid tissue.

Bacteriologic examinations of stools and of the colon were negative, but *S. paradysenteriae* was isolated from the gallbladder.

Case 3

A 7 month old Negro boy was hospitalized on November 1, 1947, after having been ill for ten days with diarrhea and fever. Spinach green stained mucous stools were passed four or five times daily; blood was never seen. Treatment with sulfaguanidin had not been successful. This child also seemed dehydrated, and the temperature was 102.2 F., the pulse was small and frequent and the heart sounds were soft. Bronchitic rales were heard over both lungs. The liver was palpable. Microscopic examination of feces disclosed an uncountable number of white blood cells but no amebae or eggs. The initial treatment in the hospital consisted of hypodermoclysis with isotonic salt solution, oral administration of succinylsulfathiazol and three-hourly intramuscular injections of 50,000 units of penicillin. On the next day the temperature did not fall below 101.3 F. On the third day a transfusion of 100 cc. of blood from the mother was given. Otologic examination (by Dr. Chateau) revealed a right otitis media, and paracentesis was performed. A lumbar puncture, performed because of slight rigidity of the neck, gave clear fluid. The urine contained red blood cells, and therefore, intravenous sodium lactate was administered by drip. Mean-

while the temperature reached 104 F. An x-ray film of the chest revealed evidence of pleuritis on the right side and several focal shadows in both lungs, which were interpreted as bronchopneumonia. Intramuscular injections of 100,000 units of streptomycin were then administered every three hours. The temperature oscillated about 104 F. until death on the eighth day of hospitalization.

Autopsy was performed (by P. H. H.) fifty minutes after death. The gross findings were as follows: Fibrinous pleuritis and bilateral small subpleural abscesses, moderate fatty infiltration of the liver, flabby kidneys with a few deeper lying red areas on the surface, redness and swelling of the mucous membrane of the colon without visible ulceration. Microscopic sections of the colon showed an almost intact superficial epithelium; only a few small foci of infiltration in the wall, with migration into the lumen of polymorphonuclear leukocytes and disappearance of the epithelium; extension very rarely of such infiltration as far as the submucosa; dilatation of a few crypts of Lieberkühn containing polymorphonuclear leukocytes.

Bacteriologic examination was made of stools from the first day of hospitalization, and of the gallbladder and a part of the colon at autopsy. The stools and the gallbladder contained *S. paradyenteriae*. Both cultures belonged to Type "W" (Flexner II). The culture of the colon was negative for *Shigella*.

DISCUSSION

It is apparent from the systematic examination of the gallbladder in the lethal cases of bacillary dysentery that the presence of *Shigella* in this organ is not of very rare occurrence. We may add that our efforts to isolate *Shigella* in 8 cases from the bile during life have failed. The papers of Tscherning¹ and of Levy² indicate that *Shigella* may persist in the gallbladder for many years. According to Lentz and Prigge,¹ Duval and Brasset (1903) and Knox and Schorer (source of reference not given) isolated *Shigella dysenteriae* from the liver of babies who died from "summer diarrhea", while Baertlein and Huwald (1914) found "Y" bacilli in the bile of young children with intestinal disorders.

Moreover, the isolation of *Shigella* from blood, spleen and urine has been recorded. Therefore, it may be concluded that *Shigella* may spread from the intestines to other organs and that this event is not as rare as has been supposed hitherto.

The dysentery bacilli in the human host can imitate the behavior of typhoid bacilli. Pot *et al.*³ reported instances of bacillary dysentery with early symptoms and signs suggesting typhoid fever, but followed some days later by a characteristic diarrhea and the results of bacteriologic and serologic examinations which revealed the true cause of the illness.

The small number of our cases does not permit an opinion regarding the failure to isolate *Shigella* from the large bowel. We cannot attribute the failure to recover the organisms in the first patient to the use of sulfonamides, and it is very improbable that this is the reason in the second patient. The inconspicuous gross and microscopic findings in the colon of these cases do not differ from those in which a positive culture resulted. In bacillary dysentery of young children very slight alterations of the colon are the rule.

Finally, we wish to stress the fact that in Curacao, in more than three years, not a single autopsy has been performed in an adult suffering from bacillary dysentery, although nearly one-fourth of the total number of deceased are examined

postmortem, and notwithstanding an extensive epidemic which prevailed in December 1944 and January 1945 (with notifications of 63 bacteriologically confirmed new cases in the week from December 4 to 10, 1944). In our opinion, a favorable influence of modern active treatment with sulfonamides cannot be denied.

SUMMARY

During the period from September 1944 to December 1947, *Shigella paradysenteriae* was isolated from the gallbladder in three lethal cases of bacillary dysentery, while simultaneous cultures from the colon were negative. During the same period, *Shigella* was isolated at autopsy 8 times from the colon but not once from the gallbladder. All of these patients were young children. An infection of the gallbladder in bacillary dysentery is not as rare as is generally supposed.

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EDITORIAL

SALMONELLA INFECTIONS IN MAN

All of the approximately 150 species of *Salmonella* known to date are pathogenic for animals or man. The genus *Salmonella* includes organisms formerly called "enteric" bacteria, the "paratyphoid" organisms and the typhoid bacillus. A few basic biochemical characteristics and an elaborate antigenic structure fitting into the Kauffmann-White-Edwards schema bind together this group of gram-negative microbes.

The clinical manifestations caused by salmonellae in man include typhoid-like fever, serious septicemia, acute or chronic enteritis or localized inflammation, the latter chiefly involving the gallbladder, urogenital organs and bones. The above clinical picture is largely dependent on the age and general immunobiologic state of the infected person. The young and the very old, the emaciated or the individual afflicted with another disease are likely to develop more serious forms of salmonellosis. Some strains, however, trend to produce one clinical picture more frequently than another; e.g., the paratyphoid organisms and *S. sendai* often produce typhoid-like fever, *S. choleraesuis*, septicemia and meningitis, and *S. give*, enteritis.

Salmonellae are transferred from man to man by direct contact, by food infected by handlers and by contaminated water and fomites; from animal to man by eating contaminated meat (chiefly pork and fowl), contaminated eggs and milk or their products, food contaminated by rodents during storage, by drinking water contaminated by animals and by handling infected animals. The exact serologic diagnosis of the strain causing the case under observation and the knowledge of the strains predominant in the given geographic area are indispensable for epidemiologic investigation. Medical laboratories usually have to be satisfied with the diagnosis of the genus while the species or type is differentiated in so-called *Salmonella* centers which render their services without any fee. The best known civilian centers in the continental United States are the Beth Israel Hospital, New York; the Agricultural Experiment Station, University of Kentucky, Lexington, Kentucky; and the Division of Veterinary Science, University of California, Davis, California; and the State Health Laboratories of Connecticut, Georgia, Maryland, Michigan and New York. According to the reports of these centers, the most frequent salmonellae encountered in persons in the United States are *S. paratyphi B*, *S. typhimurium*, *S. derby*, *S. choleraesuis*, *S. monterideo*, *S. oranienburg*, *S. newport*, *S. typhosa*, *S. panama*, *S. anatum*, *S. give* and *S. senftenberg*.

The specific therapeutic measures used in salmonellosis consist of streptomycin and of combinations of Penicillin X with soluble sulfa drugs. The testing of the susceptibility of salmonellae to antibiotics does not have to be delayed until the report on the type is received from the typing center. The general mortality in salmonellosis is 6 per cent, varying according to the physical condition of the

afflicted person and according to the strain. The mortality varies from practically zero in infections with *S. give* to 8 per cent in typhoid fever, 25 per cent in *S. choleraesuis* and *S. typhimurium* var. copenhagen infections, and nearly 100 per cent in epidemics caused by *S. havana*.

Salmonellosis, being a disease of both animals and man, can be eradicated only by the cooperation of physicians, veterinarians, public health agencies and the general public. The losses and expenditures caused by salmonellae are very high and have been estimated at \$41,000,000 per year for the United States. This sum does not include the money spent on water purification and other general sanitary measures which also contribute to the checking of such infections.

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Chicago

OSCAR FELSENFELD, M.D.

SELECTED ABSTRACTS

Q Fever Studies in Southern California. R. J. Huebner, Senior Assistant Surgeon; W. L. JELLISON, Parasitologist; M. D. BECK; R. R. PARKER, Director; and C. C. SHEPARD, Surgeon, U. S. Public Health Service, Bethesda, Maryland.

This article is one of a series of reports of the results of a cooperative study by the National Institute of Health, California State Department of Public Health and the Los Angeles County Health Department. The authors report the recovery of *Rickettsia burneti* from raw milk in 4 dairies situated in an area in which Q fever is endemic. The report covers methods of isolation of the organism by animal inoculation, serial animal passage of organism and of establishment of its identity by complement-fixation and antigenic procedures. Fertile egg technics were also used. Although rickettsiae were recovered without difficulty from raw milk, the authors report their inability to demonstrate these organisms in the blood, urine or feces from cattle in these dairies. The organisms were not recovered from possible insect and arthropod vectors. The cows shedding these organisms exhibited no clinical evidence of mastitis. This report indicates that pasteurization is an efficient procedure in the destruction of these organisms.

The authors are justifiably conservative in their present estimation of the epidemiologic significance of the findings.

This report should be read in its entirety both because of its significant addition to our present sketchy knowledge of Q fever and also because it presents in some detail the laboratory methods currently available for the isolation and identification of the causative organism.

Lansing, Michigan

H. E. CORE

Congenital Hemolytic Jaundice. The Pathogenesis of the Hemolytic Crisis. PAUL A. OWREN. Blood, 3: 231-248, 1948.

The author presents evidence pointing to an entirely new concept of the pathogenesis of the anemia in congenital hemolytic jaundice. Observations made on 6 patients showed that during the development of the acute anemia there is leukopenia, thrombocytopenia, decrease in bilirubinemia and urobilinuria, increase in serum iron, blood urea and uric acid. Examination of bone marrow at this time disclosed severe hypoplasia of erythroid elements with maturation arrest of leukopoietic and thrombopoietic elements. Recovery from the crisis was manifest by leukocytosis, thrombocytosis and finally reticulocytosis. The bone marrow showed marked erythropoietic activity and return to normal maturation of the leukocytes. The serum iron fell, and urea and uric acid figures became normal.

The author suggests that the crisis should be called "aplastic" rather than "hemolytic".

Brooklyn

LEO M. MEYER

Annotation: Suffocation by Milk Feeds. Lancet, 1: 255, 1948. *Suffocation by Milk Feeds (Letter).* C. J. POLSON and D. E. PRICE. Lancet: 1: 343, 1948.

The British press recently headlined "Babies Killed by Lumps in Dried Milk", suggesting that death was produced by aspiration of dried masses of milk. In its annotation, the *Lancet* remarked that "there does seem to have been a considerable increase in the deaths from milk suffocation; in the country as a whole the annual number is said to have increased threefold in 10 years." Polson and Price of the Department of Forensic Medicine, University of Leeds, were of the same opinion, based on what appears to be a regrettably small sample. The latter consisted of 27 infants, aged 9 months or less, certified as having died of "inhalation of vomit" in Leeds for the years 1945-1947. These represented 6 per cent, 9 per cent and 15 per cent, of all deaths of infants (excluding stillbirths) examined at the Leeds city morgue. Of the 27 babies, however, soft feather pillows were probably responsible for asphyxia in 6, and a head shawl in another case; hence, milk aspiration was probably a terminal phenomenon and cannot be held responsible for the fatalities. Seven other

babies "had been crying during the hours preceding their deaths", which may have been caused by pre-existing disease. Finally, necropsy protocols were not abstracted or were given in reference. The evaluation of aspiration of vomitus in establishing the cause of death is a constant problem to the medicolegal worker. Only careful and complete necropsy, with chemical and bacteriologic assistance, can furnish the data for an even more carefully planned appraisal.

Fort Wayne, Indiana

S. M. RABSON

The Effect of Mixtures of Sulfonamides and Urea Derivatives upon Bacterial Growth in Vitro.

D. J. TENENBERG, H. M. TSUCHIYA, W. G. CLARK, and NORA LARSON. *J. Immunol.*, **58**: 121-132, 1948.

Substances structurally related to urea were used to determine their "synergistic" action with sodium sulfathiazole on *Endamoeba coli*. In the presence of *p*-aminobenzoic acid the following, in decreasing order of activity, "synergized" sulfathiazole: O-ethylisourea-HCl, guanidine-HCl, dicyandiamide N-methyl thiourea, thiourea and urea.

In the concentrations tested, alloxan, carbromal, sodium diphenylhydantoin, methyl-urea, propadrinurea (3-propionyl-6-methyl phenyl) urea, succinamide and urethane failed to show "synergism".

Urea, thiourea, N-methylthiourea and O-ethylisourea-HCl, "synergized" sulfathiazole against sulfonamide-resistant staphylococci.

Apparently, there is a relationship between the structure of the substance and its "synergistic" activity. Substituting $=S$ for $=O$ increases the activity while substituting $=NH$ for $=S$ increases the activity further. Substitution in the amino group may or may not increase activity.

Dallas, Texas

J. H. BLACK

A New Stain Mixture: Aecto-Orcein Fast Green. N. B. KURNICK and H. RIS. *Stain Technol.*, **23**: 17-18, 1948.

The authors describe a new stain mixture which can be used with paraffin sections, but is of greatest advantage in wet tissue imprints and spreads of fixed tissue cells. It is easy to handle and may be applied to fixed or unfixed preparations.

The mixture, composed of 1 per cent orcein in 45 per cent acetic acid (27 cc.), 1 percent fast green in alcohol (3 cc.) and 2 M sodium chloride solution (2 cc.), is applied to the sections or cell spreads for a "few minutes". Nucleoli, cytoplasm, connective tissue and red blood cells are stained different shades of green, while chromatin is stained brownish-red.

Chicago

BEN FISHER

A Modification of the Waugh-Ruddick Test for Increased Coagulability of the Blood, and Its Application to the Study of Postoperative Cases. SEYMOUR B. SILVERMAN. *Blood*, **3**: 147-155, 1948.

In vitro coagulation of the blood, as measured by the Waugh-Ruddick technic, was studied pre- and post-operatively on patients undergoing several different types of surgery and on a series of non-surgical hospital patients. Increased coagulability was found to be present within twenty-four hours after surgery, and this resolved to normal within one week. The author postulates that this change may be due to liberation of increased amounts of tissue thromboplastin during surgical manipulation.

Several modifications of the original Waugh-Ruddick method (which is performed somewhat in the manner of the old Howell prothrombin time) are proposed. These include the use of a constant temperature bath and oxalated whole plasma to decrease the possibility of errors in temperature and end point.

BEN FISHER

BOOK REVIEWS

Congenital Malformations of the Heart. By HELEN B. TAUSSIG, M.D., Associate Professor of Pediatrics, Johns Hopkins University School of Medicine, and Director of the Children's Cardiac Clinic at the Harriet Lane Home of the Johns Hopkins Hospital. 618 pp., 177 figs., 46 plates. \$10.00. New York: The Commonwealth Fund; London: Oxford University Press, 1947.

Only relatively recently has anything like adequate attention been accorded the clinical aspects of congenital defects of the heart. For too long the general attitude has been that, like the primrose on the mossy bank, "a congenital heart was a congenital heart and nothing more". Fortunately, this attitude is rapidly changing. When, in 1938, Gross and Hubbard carried out the requisite preliminary studies, and then performed the first successful operation for ligation of a persistent ductus arteriosus, it brought the realization that accurate diagnosis of such conditions was imperative because certain properly selected cases could be helped by surgery. The dramatic operation for supplementing the blood going to the lungs in cases of congenital pulmonary stenosis first reported by Blalock and Taussig in 1945, and the new diagnostic data made available by the development of the catheterization technic, have given tremendous added impetus to this field. The appearance of Dr. Taussig's book on *Congenital Malformations of the Heart* comes, therefore, at a most opportune time. The book is divided into 4 parts: Part One, Physiology of the Malformed Heart and Diagnostic Principles; Part Two, Malformations Which Deprive the Body of an Adequate Amount of Oxygenated Blood; Part Three, Malformations Which Permit the Body to Receive an Oxygen Supply Sufficient for the Growth of the Individual; Part Four, Therapeutic Measures. Part One is relatively brief. Its first chapter, which purports to deal with "Embryology, Etiology, Basic Principles of Analysis, and Fetal Circulation", is most disappointing. The impossibility of covering such a sweep of territory adequately in 17 pages would seem self-evident. The embryologic background, which for a book of this sort should be meticulously worked out, is entirely inadequate.

In gratifying contrast are the other two chapters in this section, "Methods of Diagnosis" and "Cyanosis". In them one finds a straightforward and beautifully logical presentation of the factors involved in the differential diagnosis of the various types of congenital defects. Particularly noteworthy are the carefully worked out series of line diagrams analyzing x-ray findings of abnormal conditions and contrasting them point for point with the normal.

Part Two is composed of 11 chapters covering the important types of congenital defects in which cyanosis is likely to be present. As one would expect, the chapter (V) dealing with the tetralogy of Fallot is outstanding in this section. Chapter X on transposition of the great vessels is also unusually helpful on matters of importance in diagnosis, although the discussion of the developmental factors involved in the differing degrees of rotation of the arterial trunks is weak. Chapter XI on the truncus arteriosus presents a number of cases that are interesting from a clinical standpoint. The inclusion in this group, however, of cases such as those diagramed in Plates 25 and 26 in which the presence of a rudimentary pulmonary trunk clearly indicates the vessel labeled "truncus" is not an undivided truncus at all, but a large dextroposed aorta, is regrettable. It is true that functionally the aorta behaves somewhat like a truncus arteriosus, but overemphasizing this fact and ignoring the obvious anatomy and the clearly implied embryologic derivation does not help toward a real understanding of such cases. Such lapses in a work of this importance are particularly unfortunate because the book as a whole is so obviously sound and valuable that its readers will tend to accept all its statements at face value.

Chapter XIII on "Anomalies of the Venous Return" presents some interesting vascular abnormalities, several of which are of quite unusual types. One hesitates under such circumstances to make technical criticisms. It is, however, difficult to accept without protest "a left superior vena cava" (Fig. 95 and Plate 31), which in reality consists of anomalous

pulmonary veins discharging by way of a short retained portion of the left anterior cardinal and the left innominate vein, into a normal (right) superior vena cava. A left superior vena cava results from retention of the left common cardinal vein which passes diagonally across the dorsal wall of the left atrium and empties into the right atrium by way of the coronary sinus.

Part Three is made up of Chapters XV to XXV (pp. 329-534) inclusive. The chapters on the "Ductus Arteriosus" (XV) and on "Defects in the Auricular Septum" (XVI) are outstanding in this section. A good example of the effectiveness of presentation in these chapters is afforded by the x-ray films on p. 370, facing the line diagrams on p. 371, interpreting the abnormal in contrast with the normal findings. Such well selected, interpretative illustrations are more readily understandable and far more valuable than page upon page of words.

In the chapter on "Defects in the Ventricular Septum" (XVII) it is disturbing to find the vague term "high septal defect" used to designate a condition which would more accurately be covered by the anatomically and embryologically accepted designation "defect of the membranous part of the interventricular septum". One is surprised, also, to find only 2 references given at the end of this chapter dealing with a field on which there is a considerable literature.

Chapter XXI on anomalies involving the aortic arches contains some excellent illustrations of the variants in aortic arch arrangement which are of special clinical importance. In this section again, however, one misses an effective handling of the embryologic background which could do so much to make the findings more understandable.

Chapter XXII on coarctation of the aorta is well handled and well illustrated. Some of the analyses as to pressure differences, and the differences in the areas involved in cyanosis when the coarctation is combined with a patent ductus arteriosus, should prove most helpful.

One wonders a little at the amount of space devoted to dextrocardia (Chapter XXIII), which after all should not present any very startling diagnostic challenge, and to Epstein's disease (Chapter XXIV) which is exceedingly rare and which (to quote Dr. Taussig, p. 519) "is difficult, if not impossible, to diagnose clinically".

Part Four on "Therapeutic Measures" is brief, well written and authoritative. It is in such territory that Dr. Taussig is thoroughly at home and most convincing.

A reviewer, from the nature of his task, is prone to seek out and call attention to the weak points he finds. These should not, however, be allowed to assume undue prominence. In looking at Dr. Taussig's book as a whole, its minor shortcomings should not be allowed to obscure its great clinical value. It is clearly written in a simple, straightforward style and, on the whole, is well illustrated. One could wish for more documentary figures in the form of critically sharp photographs of at least a few of the more important specimens. The colored plates, to a standard pattern, are of generous size and well labeled. They would, however, be improved by carrying the color shading along the lines followed by mixing blood currents rather than showing an unnaturally abrupt change where a septal defect exists. It is a great joy to an investigator to find the references in the bibliography given in full instead of by the thoroughly annoying and time-wasting method of giving citations only by name and journal reference. In brief, Dr. Taussig has produced an unusually valuable book which no one interested in congenital defects of the heart can afford to be without. Its outstanding contributions to the clinical phases of the subject far outweigh its weakness in handling the underlying developmental processes involved.

Ann Arbor, Michigan

BRADLEY M. PATTEN

National Institute of Health Bulletin No. 189, Studies on Schistosomiasis. By WILLARD H. WRIGHT, Scientist, Director; ELOISE B. CRAM, Parasitologist (Medical); ELMER G. BERRY, S. A. Scientist; PAUL A. WARD, Parasitologist (Medical); DOROTHY TRAVIS, Junior Zoologist; RUTH E. RUE, Parasitologist (Medical); VIRGINIA S. FILES, Parasitologist (Medical); MYRNA F. JONES, Zoologist; WILLIAM B. FIGGAT, Biologist; FREDERICK J.

BRADY, Senior Surgeon; WALTER L. NEWTON, S. A. Sanitarian; S. R. WEIBEL, Public Health Engineer; HAROLD B. WARREN, Biologist; MARY LOUISE STEINLE, Biologist; MIRRIEL S. HUMMEL, Junior Chemist; M. O. NOLAN, Zoologist; ELIZABETH ROGERS MANN, Zoologist; HELEN N. CHURCHILL, Protozoologist; JOHN BOZICEVICH, Parasitologist (Medical); HELEN M. HOYEM, Zoologist; U.S.P.H.S., From the Division of Tropical Diseases National Institute of Health. 212 pp., 3 figs., 38 tables. \$.50. Washington: United States Government Printing Office, 1947.

The studies reported were carried out for the greater part at the request of the Surgeon General of the Army. Most of the results have been withheld until the present for reasons of security. The object of the studies was to evaluate: (1) the possibility of schistosomiasis becoming endemic in the United States; (2) new methods of diagnosis; and (3) protective measures both for individual use in preventing attack of cercariae on man and for large scale prevention of infection of intermediate hosts.

The question of the existence in the continental United States of snails susceptible to infection with *Schistosoma mansoni* is of particular public health importance, and a series of experiments was carried out with this point in mind. A total of 103 species and subspecies of snails was collected in 235 localities in 31 states. Twenty-seven species and subspecies were exposed to the Puerto Rican strain of *S. mansoni*. The only positive results involved a species of *Tropicorbis*, *T. havenensis*, found only in Louisiana. Subsequent to exposure, 9 specimens of this planorbid shed cercariae which were infective for mice.

Attempts to infect American snails with *S. haematobium* were wholly unsuccessful, while the African controls were infected to some extent but not in large enough percentages to be called a proper control.

When miracidia of *S. japonicum* were used, the test was not conclusive and would need to be repeated with better material. However, it would seem that *Pomatiopsis lapidaria*, which is found in Michigan and Virginia, may possibly serve as host for *S. japonicum*. *P. lapidaria* closely resembles *Oncomelania* of Asia, one of the important snails which serve as intermediate hosts for this parasite.

Experimental infection of laboratory animals with schistosomes of man yielded poor results with rats, rabbits and guinea pigs. Hamsters and mice, however, proved susceptible to *S. mansoni* and *S. japonicum*, but not to *S. haematobium*. Dogs were satisfactory as hosts for *S. japonicum*.

Studies of the effects of treatment of water on schistosome cercariae were carried out, and it was found that alum and soda ash coagulation was inadequate while filtration with diatomaceous silica and the Seitz filter was effective. Experiments with various chlorine compounds showed the cercariae to be less resistant than the cysts of *Endamoeba histolytica* and to be generally effective. Iodine compounds were generally satisfactory except where there was a high organic content with accompanying iodine demand. Copper sulphate in a concentration of 50 parts per million might have some value, but a concentration 5 to 10 parts per million could not be relied upon.

Studies on the effects of sewage treatment processes on ova and miracidia showed that the majority of ova were removed from sewage by primary sedimentation after the last addition of fresh solids. In drying sludge beds, three weeks' drying is recommended. Filtration with trickling filters is inefficient, but perhaps chlorination of the effluent would help. Activated sludge proved to be an excellent medium for hatching ova. Intermittent sand filtration is efficient with sand of 0.30 mm. effective size and a uniformity coefficient of 2.6 and with a filtering rate of 100,000 gallons per acre per day. Chlorination killed the miracidia readily, but the ova were more resistant.

Experiments to determine the protective value of chemically impregnated fabrics against penetration by schistosome cercariae were carried out using standard Army uniform material. Seventy-five chemical compounds were tested. Tests were conducted after repeated rinsing in water. The most effective chemical was N,N-diethylauramide which protected mice against *S. mansoni* cercariae through at least one hundred and forty-four

hours of rinsing. It was not resistant to soap. A measure of protection was afforded by untreated material, especially woolen serge cloth.

The last section of the report deals with a study of intradermal and serologic tests as an aid in diagnosis. The tests were made on 474 patients demonstrated to have or have had schistosomiasis japonica. The conclusion drawn was that immunologic findings were less reliable than stool examinations. It was felt that improvement in immunologic methods will have to be effected before such tests can be reliably employed in diagnosis.

The authors are to be commended for a thoroughgoing piece of work and especially for the ingenuity in developing technics necessary for carrying out the experiments.

Rochester, New York

W. S. THOMAS

The Rh Factor in the Clinic and the Laboratory. Editors: JOSEPH M. HILL, M.D., and WILLIAM DAMESHEK, M.D. 15 Contributors. 192 pp., 75 tables, 25 figs. \$4.25. New York: Grune and Stratton, Inc., 1948.

In November 1946 a group of investigators met in Dallas, Texas under the sponsorship of the Baylor University Hospital to discuss and hear discussed the various problems associated with the Rh factor. The present volume, which is a special issue of the journal *Blood*, includes many of the papers presented at this meeting, some published in their original form and others enlarged and modified subsequent to their presentation.

The meeting was opened by Dr. Philip Levine who gave a brief resumé of the discovery of the Rh factor and outlined some of the major developments that have followed in the wake of the identification of this isoantigen. The paper has been enlarged since it was originally read and now presents an excellent brief summary of the discovery of Rh and Hr, the genetic problems involved in inheritance, the classification and identification of Rh and Hr antigens and antibodies, the means of preventing immunization as well as a discussion of erythroblastosis, its diagnosis and treatment.

Dr. R. R. Race, one of the outstanding English workers in the field, outlined the identification of the different Rh and Hr subgroups by investigators in England, work which paralleled and supplemented that done in the United States. He discussed the CDE nomenclature and the English theories of gene transmission.

Drs. Joseph M. Hill and Sol Haberman presented evidence they believed pointed to the existence of a third order of Rh antibodies incapable of agglutinating or blocking red blood cells. Dr. Ernest Witebsky discussed the interrelationship between the Rh system and the AB system, and Dr. Alfonso C. Velez Orozco described the A and B factors as a possible cause of erythroblastosis. Dr. I. Davidsohn showed material indicating a correlation between the variety of Rh antibodies present in the mother's blood and the manifestations of erythroblastosis in the infant. Dr. Bruce Chown discussed the reproductive histories of a group of women immunized to the Rh factor. The treatment of erythroblastosis by substitution transfusion was described by Dr. Harry Wallerstein.

In fields other than those dealing directly with the Rh factor were the papers of Dr. William Dameshek on hemolytic mechanisms, of Dr. I. Gonzalez Guzman on the nucleolar content of certain blood cells and the banquet address of Dr. Eduardo Uribe Guerola on the history of blood transfusion in Mexico. Included in the volume but not presented at the meeting is a paper by Dr. E. E. Muirhead and others on acute renal insufficiency due to incompatible transfusion and other causes with particular emphasis on management. Missing in the volume is the excellent paper presented at the meeting by Dr. Louis Diamond in which he outlined his work on Rh antibodies, described the attempted immunization of Rh-negative individuals and reported on the incidence of immunization following blood transfusions.

The meeting in addition to being informative was a great stimulation to all who attended. It gave an opportunity for a free interchange of ideas, it permitted informal discussion of the many phases of the Rh problem and pointed the way for further investigation.

The editors in preparing the present volume have made it possible for those not fortunate enough to have been in attendance to share in the spirit of the meeting as well as in the fac-

tual material that was presented. It should be of great interest to all investigators and clinicians interested in the Rh factor.

Chicago

EDITH L. POTTER

Recent Advances in Pathology. Ed. 5. By GEOFFREY HADFIELD, M.D., F.R.C.P. (Lond.), Professor of Pathology in the University of London, Pathologist to St. Bartholomew's Hospital, formerly Examiner in Pathology in the University of London; and LAWRENCE P. GARROD, M.A., M.D., B.Ch. (Camb.), F.R.C.P. (Lond.), Professor of Bacteriology in the University of London, Bacteriologist to St. Bartholomew's Hospital, Examiner in Pathology in the Universities of Cambridge, London and Oxford. 363 pp., 60 illus. \$6.00. Philadelphia: The Blakiston Company, 1947.

In the preface to the first edition of this book the authors stated their aim "to present recently acquired knowledge of disease processes in a form useful to the student of medicine". To comply with this self-imposed task the book has been subjected to a number of changes in each of the 5 editions since 1932. It should be noted that a later edition does not bring merely the knowledge acquired since the previous edition, but that it is a revised version of the earlier edition. In the course of time some subjects have been dropped, for instance, the central nervous system, deficiency diseases, gallbladder, pancreas. More important than omission of some and additions of entirely new chapters has been the fact that the subjects presented under the old headings have been reorganized and rewritten to bring them up-to-date. All this has been accomplished with only slight fluctuations in size of the book.

The present edition has an entirely new chapter on the liver with an account of recent work on the relationship between hepatic disease and dietary deficiencies and on epidemic hepatitis. The chapter on nephritis contains the appealing, simplified classification and study of the evolution of the disease by Ellis and associates at the London Hospital. Changes have also been made in the chapters on experimental cancer and on inflammation.

It is difficult to take issue with the selection of subjects in a book of this type. Limitations of space and individual preference are the determining factors. Nevertheless, many will miss a reference to the Rh factor.

There is occasional oversight in revision, for instance, when it is stated on page 224, that "twelve years ago there was no clear etiological basis for an anemia not manifestly due to loss of blood, or to destruction of red cells or damage to the marrow by known poisons or infections".

A few minor errors have been noted. Lurie (pp. 22 and 23) is erroneously spoken of as a woman, and on page 31 lymphogranuloma "maligna" should be corrected to "malignum".

The outstanding feature of the book is the excellent and lucid presentation of difficult subjects. It is a good example of saying much in few well chosen words.

The references at the end of each chapter have been brought up-to-date.

The book will, no doubt, continue to enjoy its well-merited popularity among pathologists and physicians in other specialties who wish to keep abreast of advances in pathology.

Chicago

I. DAVIDSOHN

Tuberculosis: A Discussion of Phthisiogenesis, Immunology, Pathologic Physiology, Diagnosis and Treatment. By FRANCIS MARION POTTINGER, A.M., M.D., LL.D., F.A.C.P., Emeritus Professor of Medicine, School of Medicine, University of Southern California; Medical Director, Pottenger Sanatorium and Clinic for Diseases of the Chest, Monrovia, California. 597 pp., 105 figs. \$12.00. St. Louis: The C. V. Mosby Company, 1948.

This is a volume by an inquisitive, enthusiastic and ardent clinical student of tuberculosis who has been guided by an aphorism of Bertrand Russell, "The test of scientific truth is patient collection of facts, combined with bold guessing as to the laws binding the facts together". Pottenger, a respecter of scientific knowledge, recognizes that many state-

ments are mere opinions which must be investigated anew. Many of Pottenger's "whys" in tuberculosis are still unanswered. He possesses very little phthisiophobia since he views tuberculosis as mildly infectious and as quite susceptible to eradication. Primary infection is viewed as a vaccination which creates specific protection, and as wholly desirable, were it not for the fact that it also furnishes a focus from which future metastasis may arise. Tuberculosis is viewed as a curable disease that ends in healing in most instances when appropriate aid is rendered.

Fifty years ago when Pottenger began his practice, every phase of the disease was surrounded by ignorance and pessimism; but in spite of greater knowledge and increased optimism today, we are still only in the twilight zone of factual information. Beside our definite scientific knowledge in this disease, there is so much speculation, philosophy and dogma as to make our position on phthisiogenesis uncomfortable. We recognize as facts that the tubercle bacillus is the specific cause of tuberculosis, that the host reacts to the bacillus and the bacillus to the host. However, many factors in these reactions require further elucidation. That tubercle bacilli will infect man is a fact, but how many bacilli are necessary for infection is speculation. It is known that the immune host will withstand larger numbers of bacilli than the non-immune host, but it is not known how many bacilli the immune host can withstand or how long his protection may last. There is sufficient evidence that living bacilli remaining in the tissues after the first infection may escape from their localization in the tissues and cause reinfection, but we do not know whether reinfection would result if an equal number of bacilli entered the tissues from without or whether reinfection would result by entrance of exogenous bacilli equal in number to that producing primary infection. The relative importance of the endogenous and exogenous sources of bacilli causing reinfection is one of the most important questions in phthisiogenesis because of its epidemiologic implications.

Pottenger has attempted to remove some of the errors which have made physical examination difficult and unsatisfactory and has described his own diagnostic measures. His approach to the disease is largely immunologic and physiologic. He shows tuberculosis in its dual aspect of a metastasizing-destructive and localizing-healing disease which is the basis of Koch's observation that reinoculation is characterized by localization and healing, and which explains the chronicity of clinical tuberculosis. When the bacilli overcome resistance, spread and cause destruction, they also bring about conditions in the host which are inimical to their own existence. If the average patient with early limited infection is put on the proper hygienic regimen when tuberculosis first begins to spread, the disease will nearly always heal spontaneously. An exception to this rule is found in those who are unusually susceptible. Compression methods are discussed as to the time when they are expedient and when they are essential. Pottenger sounds an optimistic note based on epidemiologic factors, the antituberculosis program, susceptibility in the underprivileged, control of the infection-spreader and vaccination.

All in all, Pottenger impresses one that there is a general lack of all-encompassing scientific knowledge in tuberculosis and presents his fifty years of study and experience as interpretation. The interpretation of the literature and the experience of clinical tuberculosis is by an alert, studious mind. This makes the book worthwhile although weaknesses may be found which obviously cannot be avoided completely. Pottenger and the publishers, C. V. Mosby and Company, have handled this difficult and vague subject well, both materially and psychologically.

Denver

H. J. CORPER

DIFFUSE PLATELET THROMBOSES WITH THROMBOCYTOPENIA AND HEMOLYTIC ANEMIA (THROMBOTIC THROMBOCYTOPENIC PURPURA)*

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In 1925 Moschcowitz¹⁵ described a rapidly fatal case of acute anemia which by postmortem examination displayed multiple visceral hyaline thrombi within arterioles and capillaries. The thrombi were frequently surrounded by proliferating cells from the vessel wall. The illness began with weakness of the upper extremities and was followed by fever, pain in the upper extremities, pallor, skin petechiae, paresis of the left side, coma and death. Since this first report, 14 similar cases have been reported in the American literature.^{1, 2, 4, 5, 9-12, 18, 19} It is generally agreed that the hyaline thrombi are composed of agglutinated blood platelets. In presenting another such case, we should like to mention an additional associated feature previously unemphasized, namely: a diffuse proliferative glomerulitis which for the most part was not associated with platelet thromboses.

REPORT OF CASE

A 14 year old white girl was admitted to Baylor Hospital (Service of Dr. H. M. Winans) with the following complaints as given by the mother: transient paralysis of the left arm two weeks and one week before admission and again on the day before admission; jerking of the left arm two days before admission; and jaundice and muscular weakness. Transient hemiplegia had been noted by the attending physician. There was no loss of consciousness with the attacks. Except for muscular weakness since birth, there were no other pertinent historical findings.

Physical examination on admission revealed a well-developed and well-nourished subject who did not appear acutely ill. The blood pressure was 118/78 mm. Hg., the oral temperature was 99.8 F., the pulse rate was 104 and the respiratory rate was 20. Slight jaundice, anemic appearance and multiple ecchymoses over the lower extremities were noted. The patient seemed comfortable, was quiet and ate well.

Twenty hours after admission a lumbar spinal puncture was performed under avertin anesthesia. Six hours later the patient refused food and had multiple loose stools and attacks of vomiting. Soon there developed marked restlessness, dyspnea, weak and thready pulse and periodic breathing. The oral temperature then was 100 F., the pulse rate 130 and the respiratory rate varied from 26 to 36. The immediate condition improved after a direct transfusion of 240 cc. of blood. Later, she developed abdominal pain, nausea and vomiting and had multiple bloody liquid stools.

Throughout the remainder of the patient's course (eight days), the temperature varied between 100 F. and 103 F., the pulse rate varied between 100 and 130 and the respiratory rate fluctuated between 24 and 40. Nausea and vomiting persisted throughout, as did bleeding from the bowel and vagina. Subsequently, the following manifestations fluctuated in severity: vomiting of blood, bleeding from the gums, paresthesias of the face and region of hips, weakness of the right arm, irrational interludes, weak and irregular pulse, dyspnea and tachypnea, headaches, restlessness, abdominal pain, pain in the back and legs, generalized aches and drowsiness.

* Received for publication, February 27, 1948.

Terminally, there was marked mental deterioration. Whereas the patient was described as a neat and orderly individual in health, she became demented, failed to recognize others and, while bathed in the loose, bloody stools, she played with the excreta in the manner of a highly regressed and psychotic patient.

Five blood transfusions, totaling 1390 cc., were given. On the ninth day a splenectomy was performed, with the realization that she was a poor operative risk. The patient expired four hours later in a shock-like state.

Table 1 contains the hematologic data. Severe anemia was present throughout the hospital course. The findings of typical hemolytic crisis were observed.

TABLE 1
SUMMARY OF HEMATOLOGIC DATA

	DAY OF HOSPITALIZATION			
	1st	2nd	4th	8th
Erythrocytes, millions per cu. mm.	2.7	3.75	2.65	2.29
Hemoglobin, grams per 100 ml. blood	8.3	10.2	8.2	6.4
Mean corpuscular volumes, cubic microns		89.5		93.4
Mean corpuscular hemoglobin, micro-micrograms		30.4		27.9
Mean corpuscular hemoglobin concentration, grams per 100 ml.		34.0		29.0
Mean diameter of erythrocytes, microns		7.24		7.24
Mean thickness of erythrocytes, microns		2.01		2.2
Volume thickness index of erythrocytes		1.19		1.23
Reticuloeytes, per cent of erythrocytes	38.4		22.2	51.4
Fragility of erythrocytes, Beginning hemolysis, per cent saline		.42		.55
Complete hemolysis, per cent saline		.28		.36
Icterus index			26.0	30.0
Leukoocytes per cu. mm.	13,950		22,800	10,500
Platelets per cu. mm.	87,555		229,600	119,080
Uric acid, mg. per 100 ml. blood		11.4		11.1

The anemia was of normocytic-normochromic type and was associated with spherocytosis, increased red cell fragility to hypotonic salt solutions, hyperbilirubinemia, increased reticulocyte count and hyperuricemia. Nucleated red cells were noted in the peripheral blood smears (average 12 per cent). Leukocytosis and a prominent left shift in the hemogram were associated with the hemolysis. Thrombocytopenia was demonstrated on the second day after bleeding from the bowel began and persisted in moderate severity throughout the subsequent course.

The spinal puncture yielded a clear fluid with 4 cells per cu. mm., 40 mg. of protein per 100 ml. and a normal sugar concentration. Many red blood cells were seen microscopically in urine samples.

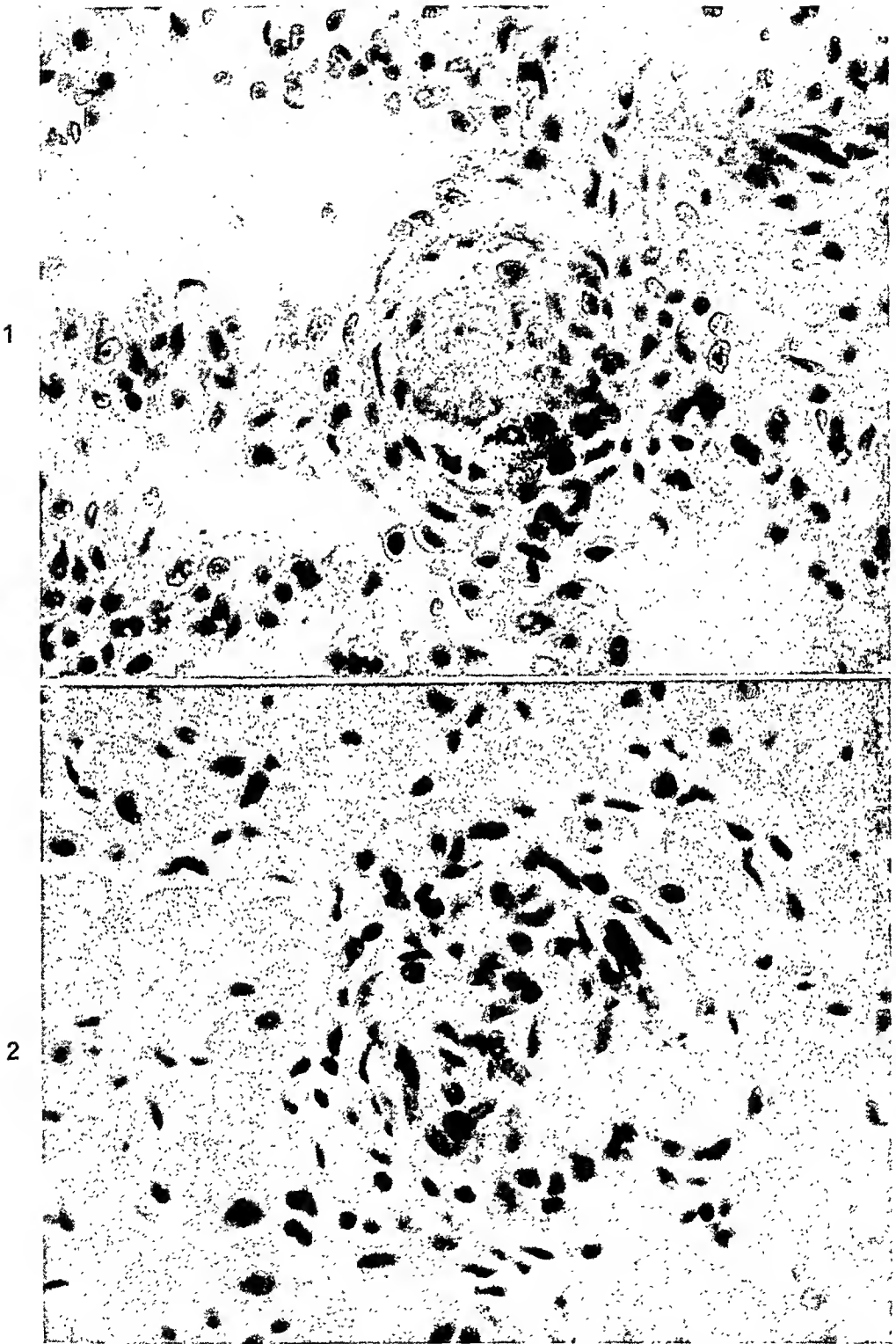


FIG. 1. Section of lung platelet thrombus which is slightly granular. Endothelial proliferation is minimal here. The thickening of alveolar walls due to increased cellularity can be seen. At the extreme right of the field there is a megakaryocyte.

FIG. 2. A hyaline thrombus in small vessel in myocardium. The surrounding proliferated endothelial cells are evident. Scattered lymphoid cells are seen in the vicinity. The myocardial fibers are granular and show an occasional vacuole.

AUTOPSY FINDINGS

Gross Examination

The recent splenectomy incision was dry, the skin was light yellow in color and ecchymoses surrounded venipuncture marks in the arms. The lungs were free. The right pleural space had about 250 cc. of blood-tinged fluid, the left space about 100 cc. of similar fluid. The pleural surfaces were smooth and exhibited multiple petechiae and ecchymoses. On section the parenchyma displayed scattered multiple hemorrhagic foci not exceeding 1 cm. diameter. A thin, frothy, serosanguineous fluid exuded on pressure.

The heart weighed 220 Gm. The muscle was flabby, pale and grayish brown in color. Multiple ecchymoses were found on the epicardial and endocardial surfaces. The valves were normal.

The peritoneal cavity contained about 200 cc. of bloody fluid but no clotted blood was noted. About 100 cc. of blood was found in the splenic bed. The liver showed prominent lobular markings. Ecchymoses were noted in the tail of the pancreas and scattered petechiae on the gastric mucosa. The intestinal contents had a reddish hemoglobinic tint and numerous petechiae were found in the mucosa of the small and large bowel. An ulcer of the sigmoid colon measured 1 cm. in diameter. The abdominal lymph nodes were slightly enlarged.

The kidneys were moderately swollen, the parenchyma pale and cloudy, and multiple petechiae were noted in the pelvic mucosa. The uterine cavity had 7 cc. of blood clot. Grossly the brain was moist but not remarkable in appearance.

Microscopic Examination

Multiple hyaline thrombi were seen in smaller blood vessels of the *myocardium* (Fig. 2) and pericardium. The thrombi were of light pink color in sections stained with hematoxylin-eosin, were usually smooth and homogeneous but at times slightly granular. Almost every thrombus was partly or completely surrounded by proliferated endothelial cells. These cells had elongated nuclei which in some instances had a folded nuclear membrane. The chromatin was fine, well dispersed and displayed an occasional chromatin knot. The cytoplasm was delicate, faint and frequently drawn out. No intercellular fibers were noted. Within the smaller vessels the endothelial cells tended to form concentric layers. In the larger affected vessels, mostly venules, the proliferating endothelial cells formed matted clusters that appeared to have invaded the thrombus. At times the thrombus appeared to have been largely replaced by these small concentric masses of endothelial cells. Venules, capillaries and perhaps precapillary vessels appeared to be the main vessels occluded by the hyaline thrombi. In a low power field (magnification $\times 50$), from 8 to 12 thrombi were seen within myocardial vessels. Myocardial interstitial edema was prominent, and a scattered lymphocytic infiltration between the thrombi gave the appearance of interstitial myocarditis. The myocardial fibers were intact and the sarcoplasm was moderately granular and slightly vacuolated.

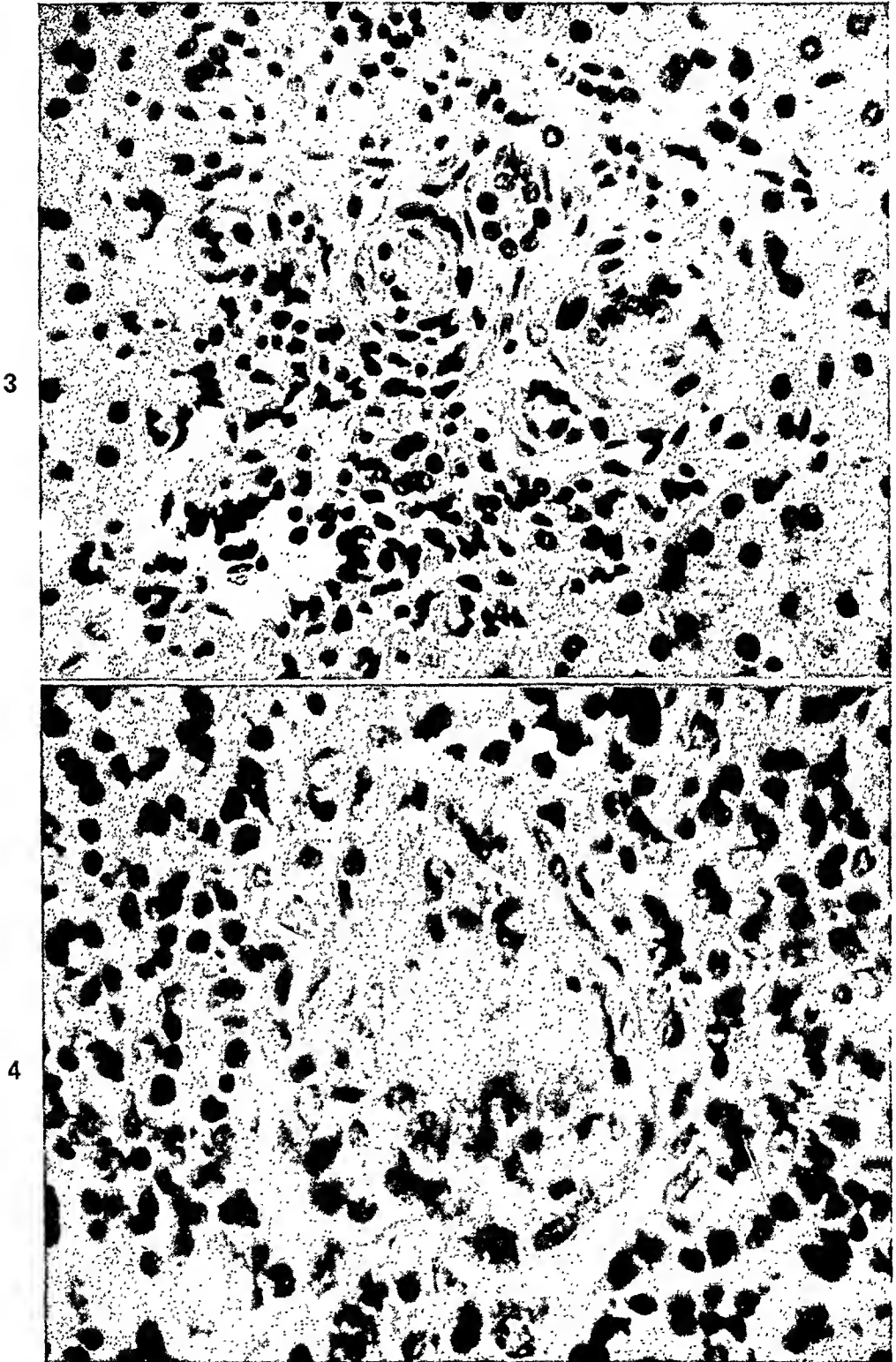


FIG. 3. A portal area in the liver showing hyaline thrombi of small vessels, slight endothelial proliferation and marked "round cell" infiltration of the connective tissue. The bile duct is intact. The parenchymal cells are granular.

FIG. 4. A hyaline thrombus and endothelial proliferation in a splenic venule.

The sections of *lung* demonstrated diffuse pulmonary edema and scattered hyaline thrombi in smaller blood vessels, and most of these were surrounded by masses of proliferated endothelial cells (Fig. 1). The venules were prominent, but the alveolar wall capillaries were less prominent. The alveolar walls were slightly thickened by a cellular infiltration, which included lymphoid cells, polymorphonuclear leukocytes, frequent megakaryocytes and other cells resembling endothelial cells.

The *liver* revealed frequent hyaline thrombi in vessels of the portal areas and again endothelial proliferation was noted. The connective tissue about these vessels showed prominent infiltration by lymphoid cells (Fig. 3). There was no excess of connective tissue. The vessels which contained the thrombi appeared to be smaller veins and capillaries. The bile ducts were normal. A slight diffuse cellularity within the sinusoidal system (small cells with dark round nuclei, cells with larger vesicular nuclei and polymorphonuclear leukocytes) suggested extramedullary hematopoiesis. There was moderate passive hyperemia with central atrophy of the lobule. The parenchymal cells had a granular cytoplasm and the nuclei frequently revealed evidence of glycogen accumulation.

The *spleen* (surgical specimen) also revealed many hyaline thromboses of smaller vessels, mostly in venules, and endothelial proliferation was again evident (Fig. 4). The follicles were large and had prominent "germinal centers". The sinusoids were well filled with red blood cells and moderate reticulo-endothelial hyperplasia was present. No erythrophagocytosis was seen.

A *lymph node* was the seat of hyaline thrombosis and endothelial proliferation. The lymphoid structure was hyperplastic and the medullary sinuses were filled with various cells (macrophages, lymphocytes, plasma cells and few polymorphonuclear leukocytes).

The *kidney* showed a prominent proliferative glomerulitis involving every glomerulus seen. The endothelial cells blocked the capillaries, the two layers of Bowman's capsule were fused in many instances (Fig. 5). Most glomeruli were without hyaline thrombi but some had the same type of hyaline thrombi previously mentioned. Thrombosis and endothelial proliferation were also noted in interstitial vessels, particularly in veins of the medulla. The cytoplasm of cells lining the proximal segment was very granular and contained scattered fine brownish granules (hemosiderin). The distal segments frequently contained granular and hyaline casts. Scattered collections of lymphocytes were found in the interstitial tissue.

Hyaline thrombi were found in sections of the small and large *bowel*. The colon had a recent infarct of its mucosa and submucosa. This area revealed coagulative necrosis, diffuse recent hemorrhages, ulceration, marked edema and early suppurative inflammation (Fig. 6).

Sections of *brain* (cortex and brain stem) showed multiple hyaline thrombi but endothelial proliferation was minimal in these areas. Nerve cell bodies displayed scattered pyknosis and loss of Nissl substance, while fiber tracts showed large vacuoles. The meninges had a moderate lymphocytic infiltration.

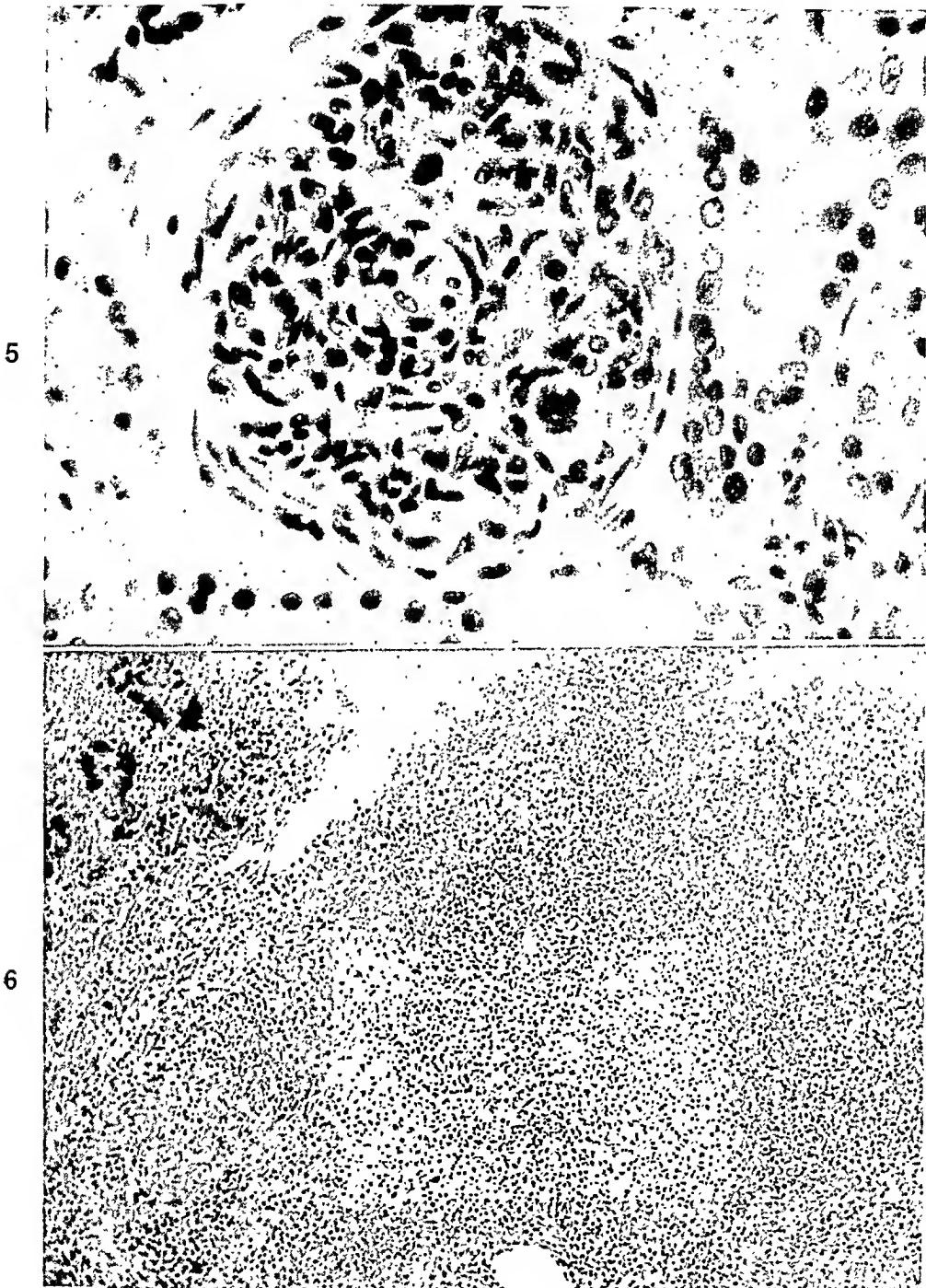


FIG. 5. Endothelial proliferation of a glomerulus without platelet thrombi is shown. Notice the fusion of the visceral and parietal portions of Bowman's capsule.

FIG. 6. A portion of the infarct of the sigmoid colon showing necrosis, ulceration and marked infiltration by polymorphonuclear neutrophilic leukocytes and hyaline thrombi.

DIAGNOSES

Clinical: Acute hemolytic anemia and thrombocytopenic purpura.

Anatomic: Platelet thromboses and endothelial proliferation of all organs; diffuse glomerulitis, proliferative type; interstitial pneumonitis, mild and diffuse; focal inflammation of meninges, myocardium, liver, kidneys, intestines, lymph nodes; recent infarct, mucosa of sigmoid colon; jaundice; parenchymatous degeneration of myocardium, liver and kidneys; passive hyperemia and edema of lungs; hydrothorax, bilateral; passive hyperemia and centrilobular atrophy of liver; recent splenectomy wound.

COMMENT

Reviews of the literature and discussions of the clinicopathologic features of this peculiar thrombotic disease, associated with thrombocytopenia and anemia, have been presented by Fitzgerald, Auerback and Frame¹⁰ and by Singer, Bornstein and Wile.¹⁸ The outstanding clinical features include a febrile and rapidly fatal course with associated thrombocytopenic purpura, severe anemia with reticulocytosis, jaundice, nucleated red cells in the peripheral blood, peculiar mental and neurologic patterns, and finally, profound coma. Leukocytosis and "left shift" have at times reached "leukemoid" proportions.¹³ Eighty per cent of the patients have been females and the average age has been 23.8 years (range from 9½ to 50 years). The diffuse small vessel thromboses and tiny visceral infarcts have offered a direct explanation of much that has been noted clinically.

The two outstanding hematologic features have been thrombocytopenia and anemia. The "sweeping-up" effect of the thromboses on the blood platelet concentration has been considered as the cause of the thrombocytopenia.⁷ An attempt by Bernheim⁴ to demonstrate the presence of platelet antibodies (platelet agglutinin) was not successful. It seems apparent that the anemia is usually of hemolytic type. In the present reported case the hemolytic anemia was associated with spherocytosis. Leukopenia has been reported only once.

The combination of hemolytic anemia and thrombocytopenia naturally leads to the consideration of the role of the spleen in this disorder. Splenectomy has been performed on three patients including our patient, without altering the clinical course. It must be emphasized, however, that in all three instances the patients were in a terminal moribund state when the operation was performed as a final desperate measure. It, therefore, appears that splenectomy has not received a fair trial; and it remains to be seen whether early removal of the spleen will alter the ultimate course.

Acute diffuse proliferative glomerulitis, similar to proliferative glomerulonephritis,³ has not been described previously in this entity. However, Carter⁵ noted a few enlarged glomeruli with increased cellularity. In the present case the glomerulitis was seldom associated with hyaline thrombi. It seems, therefore, that the glomerular endothelial proliferation was, for the most part, not directly related to the hyaline thromboses. In the lungs a similar dissociation between thrombosis and endothelial proliferation seemed to exist. Trobaugh, Markowitz, Davidson and Crowley¹⁹ noted swelling of capillaries without

thrombosis. These findings appear to be at variance with the more common belief that the endothelial proliferation is strictly secondary to the thrombosis. It appears unlikely that such a diffuse glomerular proliferation could possibly represent previously "organized" thrombi. Therefore, unless one postulates the existence of two diseases in our patient, the entity of Moschcowitz and glomerulonephritis, it must be considered that the factor responsible for the thrombotic disease was also able to stimulate endothelial proliferation without producing local thrombosis (glomeruli and lungs).

The focal "round cell" inflammatory response noted in the viscera (meninges, heart, pericardium, alveolar walls, liver, kidney and lymph node) in the present case is not the type of inflammation usually found in conjunction with acute infarcts. Moreover, this inflammatory response was often found in the vicinity of thrombosed vessels. The disparity between the thrombosis and endothelial proliferation, plus the nature of the focal inflammatory response where no infarcts are evident, are factors that are not completely consistent with the prevailing view which contends that the endothelial proliferation is secondary to the thrombosis and that the inflammatory changes result from small infarcts. On the contrary, the above findings appear somewhat more consistent with the idea of primary vascular damage, first suggested by Altschule.¹ It, therefore, seems that the evidence for and the evidence against the idea of primary vascular injury are not conclusive at present. The same may be mentioned concerning the role of the spleen and the role of antigen-antibody mechanisms. In view of what is known about hypersensitivity and hemolytic anemia,¹⁷ hypersensitivity and proliferative glomerulitis,⁶ hypersensitivity and various types of vascular injuries,¹⁷ certainly the antigen-antibody explanation seems attractive.

Two terms have been proposed for this entity, "thrombotic thrombocytopenic purpura"¹⁸ and "thrombocytic acroangiothrombosis".¹⁰ No instance has yet been reported without a severe anemia. In the patients studied, most of the evidence favors a hemolytic type of anemia. To be sure, in the present case hemolysis was severe and of such proportion as to lead to spherocytosis.⁸ Since the anemia seems to be an integral part of the disease, it perhaps should receive more consideration in the designation of the disease. For this reason we have used the admittedly cumbersome designation, diffuse platelet thromboses with thrombocytopenia and hemolytic anemia.

The clinical similarity between this disease and other conditions whose basic pathologic change involves visceral blood vessels ("visceral angiitis")¹⁴ has been frequently expressed. This similarity and the clinical similarity to thrombocytopenic purpura of Werlhof and the fact that no antemortem diagnosis has as yet been reported naturally lead to a feeling of insecurity concerning the true incidence of this condition. It may not be as rare as the small total of only 16 reported cases would seem to indicate.

SUMMARY

A report has been presented of a patient who displayed diffuse platelet thromboses with thrombocytopenia and hemolytic anemia. The clinical course and pathologic findings corresponded with prior descriptions of this entity. The

main clinical findings were: rapidly fatal febrile disease in a 14 year old girl associated with a bizarre and vacillating neurologic picture, marked mental deterioration, bloody diarrhea, hematuria, hemolytic anemia and thrombocytopenia. Splenectomy, performed when the patient was in a comatose state, was followed by fatality. Postmortem examination revealed mainly multiple hyaline thromboses of small visceral vessels, proliferative glomerulitis, focal visceral inflammation and mucosal infarction of the large bowel. The disparity between thrombosis and endothelial proliferation constituted an obstacle to the interpretation that endothelial proliferation is strictly secondary to the thromboses. The hemolytic nature of the anemia has been emphasized. In the present lack of understanding of the cause of this entity, it is believed that a descriptive designation of the disease should embody both important hematologic features, namely, thrombotic thrombocytopenia and hemolytic anemia.

Acknowledgment. We wish to thank Dr. C. King for the photomicrographs.

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INTRAGROUP INCOMPATIBILITY DUE TO THE hr" FACTOR*

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Since the original publication by Mourant,¹ no other report has appeared of hr" sensitization with demonstrable antibodies in the serum of the sensitized individual. We have recently encountered intragroup transfusion reactions in three individuals of type Rh₂Rh₂, and in the serum of one of these persons a moderately strong anti-hr" agglutinin was found. Since this is only the second instance in which such an antibody has been found, it seemed worthwhile to report it.

REPORT OF CASE

The patient, a 31 year old white female, was admitted to the Mercy Hospital for study because of incompatibility to blood of the homologous group.

Since 1933, this patient had had 8 pregnancies. The first pregnancy in 1933 ended at two and one-half months when she aborted at home. The second pregnancy ended at seven months with the delivery of a child who is living and well. While on the delivery table, the patient was given a pint of blood. After the needle was withdrawn, the patient had chills and fever lasting thirty minutes. Before discharge from the hospital she was given another pint of blood which again caused chills and fever, beginning five minutes after the needle was withdrawn from her vein. The third pregnancy in 1936 resulted in birth of a living child at seven months of pregnancy, delivery taking place at another hospital. This time the patient was given no transfusions. In 1937 the patient had another seven month pregnancy with delivery at a different hospital. The forceps "slipped after being applied to the baby's head at delivery". This child died at eight months. The patient was again given a pint of blood on the delivery table and another pint six days later. Both of these transfusions caused reactions within five minutes after the transfusions were terminated, and each reaction lasted forty-five minutes. In 1938 the patient had a two month abortion. She required cervical dilatation and uterine curettage and was given a pint of blood before the procedure. She had a reaction which lasted about an hour. Later, when it was desired to give another pint of blood, difficulty was encountered in finding a compatible donor, and as a result, the transfusion was not given. In 1940 following a seven month pregnancy, another transfusion was given which resulted in a reaction lasting forty to forty-five minutes. The seventh pregnancy in 1942 also terminated at seven months. The patient was given a pint of blood on the day before leaving the hospital, and a reaction consisting of chills and fever again occurred after the transfusion had been completed. Another transfusion seemed indicated, but was not given when it was found impossible to match any blood with the patient's serum. The eighth and last pregnancy ended at the eighth month. Again the patient required treatment for anemia, but no blood compatible with her serum could be found. Therefore, she was placed on liver and iron therapy instead.

In the fall of 1947 the patient presented herself for treatment because she had menstruated 17 times during the previous nine months. A cervical dilatation and uterine curettage were done on October 4, 1947 at another hospital. The patient was found to be anemic, but no blood transfusion was given because the patient's serum did not match with blood of her own group. The patient was then referred to us for further study and for the selection of a compatible donor for blood transfusion.

* Received for publication, March 29, 1948.

Examination of the patient's blood showed her to belong to group O, type M, type Rh₂,* and we readily confirmed the presence in her serum of an irregular agglutinin which clumped other blood of group O, but did not clump the patient's own red cells. The fact that the patient was Rh positive ruled out the possibility that we might be dealing with an anti-Rh₀ agglutinin, while the fact that the patient belonged to type M indicated that the patient's serum did not contain an anti-M agglutinin. Moreover, in tests on a random series of about 100 blood specimens only 3 or 4 bloods were encountered which gave no clumping with the patient's serum. This suggested that we might be dealing with an anti-hr'' agglutinin; and the patient's blood was, therefore, tested for the hr'' factor with the aid of serum kindly provided by Dr. Mourant. The patient did prove to be type Rh₂Rh₂, and further tests revealed that every blood that failed to clump in the patient's serum also belonged to type Rh₂Rh₂. The antibody in

TABLE 1
TITRATION OF ANTI-HR'' AGGLUTININ IN PATIENT'S SERUM

METHOD USED	TEST CELLS	REACTION ACCORDING TO DILUTION OF PATIENT'S SERUM						
		Un-diluted	1:2	1:4	1:8	1:16	1:32	1:64
Agglutination	ORh ₁ rh	++±	++±	+±	+±	±	—	—
	ORh ₂ rh	++±	++	+±	±	±	—	—
	ORh ₂ Rh ₂	—	—	—	—			
Conglutination*	ORh ₁ rh	++±	++	++	+±	+±	±	tr.
	ORh ₂ rh	++±	+±	++	+±	+	±	tr.
	ORh ₂ Rh ₂	—	—	—	—			

* In acacia (cf. Wiener, Hurst and Handman⁵)

the patient's serum was titrated against a number of blood specimens, and an illustrative protocol is given in Table 1. It will be seen that the titers by the agglutination and conglutination methods did not differ significantly, so that we were dealing with an agglutinin of specificity hr''. The possibility existed that in addition to the hr'' agglutinin the patient's serum might also contain an anti-rh' agglutinin.⁷ This could be determined only by absorption tests, but in order to conserve the patient's serum which promised to be useful as a diagnostic reagent, absorption tests were not carried out.

Since the patient belonged to group O, her serum could only be used to test group O individuals, unless the interfering alpha and beta agglutinins were absorbed or neutralized. Owing to the lack of suitable controls of types A₁Rh₂Rh₂ and BRh₂Rh₂, it has not yet been possible to remove these interfering agglutinins from the serum, so that up to now all studies have been confined to bloods of group O. In Table 2 are presented the results of such studies on two small series of Caucasian and Negroid individuals. The frequency of type

* For nomenclature of the Rh-Hr blood types, see Wiener.^{4, 9}

Rh₂Rh₂ individuals found does not differ significantly from the expected value. (The expected value of Rh₂Rh₂ individuals among Caucasians is 3 per cent, among Negroids, 2.5 per cent.)

With the aid of the patient's serum and also the anti-hr'' serum of Mourant we were able to obtain a panel of donors of group O, type Rh₂Rh₂, compatible with the patient's serum. In the meantime, however, the patient's blood count rose, and the need for another transfusion no longer existed; therefore, no further transfusions have been given to date. Should other transfusions be required, these can now be given without the danger of additional hemolytic transfusion reactions.

COMMENT

There is reason to believe that instances of sensitization to the hr' and hr'' are more common than is generally realized.^{2, 3, 5} Thus, in another paper a series of such cases will be reported by one of us.⁶ Fortunately, agglutinogens hr' and hr'', unlike Rh₀, are poor antigens, so that the frequency of sensitized individuals

TABLE 2

INCIDENCE OF hr''-NEGATIVE INDIVIDUALS AS DETERMINED WITH AID OF PATIENT'S SERUM

RACE	NUMBER OF INDIVIDUALS TESTED	NUMBER OF INDIVIDUALS OF TYPES SPECIFIED								
		rh	Rh ₁ Rh ₁	Rh ₁ rh	Rh ₂ Rh ₂ *	Rh ₂ rh	Rh ₁ Rh ₂	Rh ₀	rh'	rh''
Caucasian	84	19	24	21	4	6	9	1	0	0
Negroids	31	1	0	6	1	5	1	17		

* hr''-negative individuals

is relatively low, and those who do become sensitized produce antibodies of low titer only. As a rule, the antibodies are not demonstrable in tests *in vitro*, and when transfusions of hr''-positive blood are given, the hemolysis is incomplete in conformity with the low potency of the antibodies.^{5, 6} The presenting symptoms are chills and fever, and such cases might well be passed off as pyrogenic reactions unless the slight hemolysis occurring is detected by comparing the pre- and post-transfusion blood plasma. The present case is exceptional in that the antibodies were potent enough to be demonstrable in tests *in vitro*. But even in this case the titer of the antibodies was relatively low, which explains why the patient was able to survive the reactions she had after the many transfusions which she received.

SUMMARY

An instance is presented of intragroup incompatibility in a patient of type Rh₂Rh₂ due to sensitization to the hr'' factor. In the patient's serum anti-hr'' agglutinins of sufficient potency were present for use as a diagnostic reagent for subtyping bloods of type Rh₂. This is the first example of anti-hr'' agglutinins to be reported since the original report of Mourant.

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ANTITHROMBIN ACTIVITY OF STORED PLASMA*

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Modifications of the coagulability of stored plasma have received considerable attention. Quick⁶ in 1943 showed that the diminution of prothrombin activity during storage of oxalated human plasma is due to a progressive decrease in concentration of a factor designated as "component A" and more recently renamed "labile factor".⁹ This finding has been repeatedly confirmed.¹⁻⁴ The results presented in this note suggest that another modification, occurring in oxalated human plasma during storage, may also contribute to the apparent decrease of its prothrombin activity.

In the course of studies on the effect of storage on some plasmatic clotting factors, it was found that antithrombin activity of oxalated human plasma is definitely increased after one month of storage. Further work indicated that this increase is less pronounced in human plasma made incoagulable by the addition of sodium citrate or by passage through the resin Amberlite, in which the decrease of prothrombin activity during storage is slow and moderate. Quick⁷ in 1946 reported that the prothrombin time actually decreases during the first few days in blood decalcified with 0.128 M sodium citrate, but not if 0.2 M sodium citrate is used.⁶ These observations suggested a relationship between apparent fall of prothrombin level, increase of antithrombin activity of stored plasma and type of decalcifying agent used. The problem was further investigated with studies on dog and rabbit plasmas in which, irrespective of the method of decalcification employed, the diminution of prothrombin activity during storage is much less pronounced.

METHODS

Oxalated or citrated plasmas were obtained by adding 1 volume of 0.1 M solution of sodium oxalate or citrate to 9 volumes of blood obtained by venipuncture and centrifuging at 3000 r.p.m. for ten minutes; "Amberlite" plasma by centrifugation from blood decalcified with the resin, Amberlite, according to the technic previously described.¹⁰ All plasmas were pipetted into tubes coated with Silicone (methyl-chloro-silone, Dri Film 9987, General Electric Company, Schenectady, N. Y.) and stored in the refrigerator at 4 C.

Prothrombin activity was determined with the one-stage method of Quick using a calcium-free thromboplastin.¹⁰ Antithrombin activity was estimated by incubating equal volumes of thrombin and plasma to be studied in water bath at 37 C. for variable periods of time (from one to five minutes). The clot formed was removed by wrapping it about a glass rod coated with collodion.

* This work was supported by a grant from the United States Public Health Service. Received for publication, March 1, 1948.

After the required period of incubation, 0.1 cc. of the mixture was added to 0.2 cc. of normal oxalated plasma, homologous in species to the plasma tested, and the clotting time recorded. "Full strength" thrombin was prepared according to the technic of Quick⁵ from oxalated human plasma and diluted to the concentration required with distilled water. This technic prevents possible errors due to changes in reactivity of fibrinogen during storage, although we have found in preliminary work that fibrinogen obtained from human plasma stored

TABLE 1

DIMINUTION OF PROTHROMBIN ACTIVITY OF HUMAN, DOG AND RABBIT STORED PLASMA DECALCIFIED WITH SODIUM OXALATE OR CITRATE OR WITH PASSAGE THROUGH AMBERLITE. EACH FIGURE INDICATES THE PROTHROMBIN TIME IN SECONDS AND REPRESENTS THE AVERAGE TIME OF SEVERAL EXPERIMENTS

Source and Kind of Plasma	PROTHROMBIN TIME IN SECONDS						
	Days of Storage						
	0	5 days	10 days	20 days	25 days	30 days	40 days
Human							
Oxalated plasma*	12	22	31.5	38	44	48	59
Citrated plasma†	11	14	18.5	22	26	31	36
Amberlite plasma‡	12.5	14	17.5	23	25	31	36
Dog							
Oxalated plasma	6	7	7	7.5	8.5	9.5	11.5
Citrated plasma	5.5	6	5.5	6	6	7	8
Amberlite plasma	6.5	7	6.5	7	7	8.5	10
Rabbit							
Oxalated plasma	6	6.5	7	7	8	8.5	9
Citrated plasma	6	6	6	6	6	6.5	7
Amberlite plasma	6.5	6.5	6.5	6.5	7	7.5	8

* Nine volumes of blood were added to 1 volume of 0.1 M sodium oxalate and plasma obtained by centrifugation.

† Nine volumes of blood were added to 1 volume of 0.1 M sodium citrate and plasma obtained by centrifugation.

‡ Whole blood was decalcified by passage through Amberlite and plasma obtained by centrifugation.

for thirty days is still acted upon normally by thrombin at full strength or at lower concentrations.

RESULTS

As shown in Table 1, oxalated human plasma gradually loses prothrombin activity during storage. This progressive decrease is slower and less evident for citrated and "Amberlite" human plasmas and even less pronounced in dog and rabbit plasmas, irrespective of the decalcifying agent used. The change in antithrombin activity is shown in Table 2 and Figure 1. Fresh human, dog and rabbit oxalated plasmas exhibit a greater inhibitory effect on thrombin than citrated or "Amberlite" plasmas. In storage the antithrombin activity increases

rapidly and markedly in oxalated human, dog and rabbit plasmas and in a slower and less pronounced way in citrated and "Amberlite" plasmas. It is, however, to be noted that absolute values obtained for dog and rabbit plasmas are con-

TABLE 2

THE ANTITHROMBIN ACTIVITY (CLOTTING TIME IN SECONDS) OF FRESH AND STORED OXALATED, CITRATED AND AMBERLITE PLASMA OF MAN AND DOG*

TYPE OF PLASMA AND PERIOD OF INCUBATION	SOURCE AND AGE OF PLASMA									
	Human Plasma					Dog Plasma				
	Fresh	5 days	10 days	20 days	30 days	Fresh	5 days	10 days	20 days	30 days
	<i>clotting time in seconds</i>					<i>clotting time in seconds</i>				
Plasma incubated one minute										
Oxalated	14	19	25	31	47	13	13	14	14	22
Citrated	12½	13½	14	16	26	11	11	11	10½	12½
Amberlite	8	9	9	9½	14	8½	8	8½	9	10
Plasma incubated two minutes										
Oxalated	21	39	52	75	170	26½	27	27	27	39
Citrated	14½	15½	16½	19½	32	13	14	14	15	20
Amberlite	10	11	11	12	19	12	12	13	12	15½
Plasma incubated three minutes										
Oxalated	62	92	127	290	770	49½	50	50	51	68
Citrated	16	17½	19	23	39	17½	19	20	21	27
Amberlite	13½	13½	14	14	28	18½	19	19	19	25
Plasma incubated four minutes										
Oxalated	457	597	840	†	†	90	92	92	94	125
Citrated	21½	23	24	28	48	25	25	27	28	35
Amberlite	21	24	26	26½	40	29	29	30	28	38½
Plasma incubated five minutes										
Oxalated	†	†	†	†	†	225	235	230	240	290
Citrated	28½	30	32	36	62	34	34	36	37	45
Amberlite	34	35	37	37	51	52	54	53	55	62

* The antithrombin activity of plasma was tested as follows: One volume of plasma was mixed with 1 volume of thrombin (of such potency that 0.1 cc. clotted 0.2 cc. of fresh oxalated plasma in three seconds) and incubated at 37 C. The fibrin clot was removed. At specified intervals, 0.1 cc. of the thrombin-plasma mixture was added to 0.2 cc. of fresh plasma (source of fibrinogen) and the coagulation time was observed.

† No clotting in one hour.

sistently lower than for human plasma. Preliminary experiments showed that no increase of antithromboplastin activity occurs in stored plasmas.

DISCUSSION

The increase of antithrombin activity observed during storage of plasma appears to be dependent on the type of decalcifying agent used and not directly

related to the decrease of the prothrombin level itself. It is, therefore, well pronounced in oxalated plasma, both in human plasma which rapidly loses

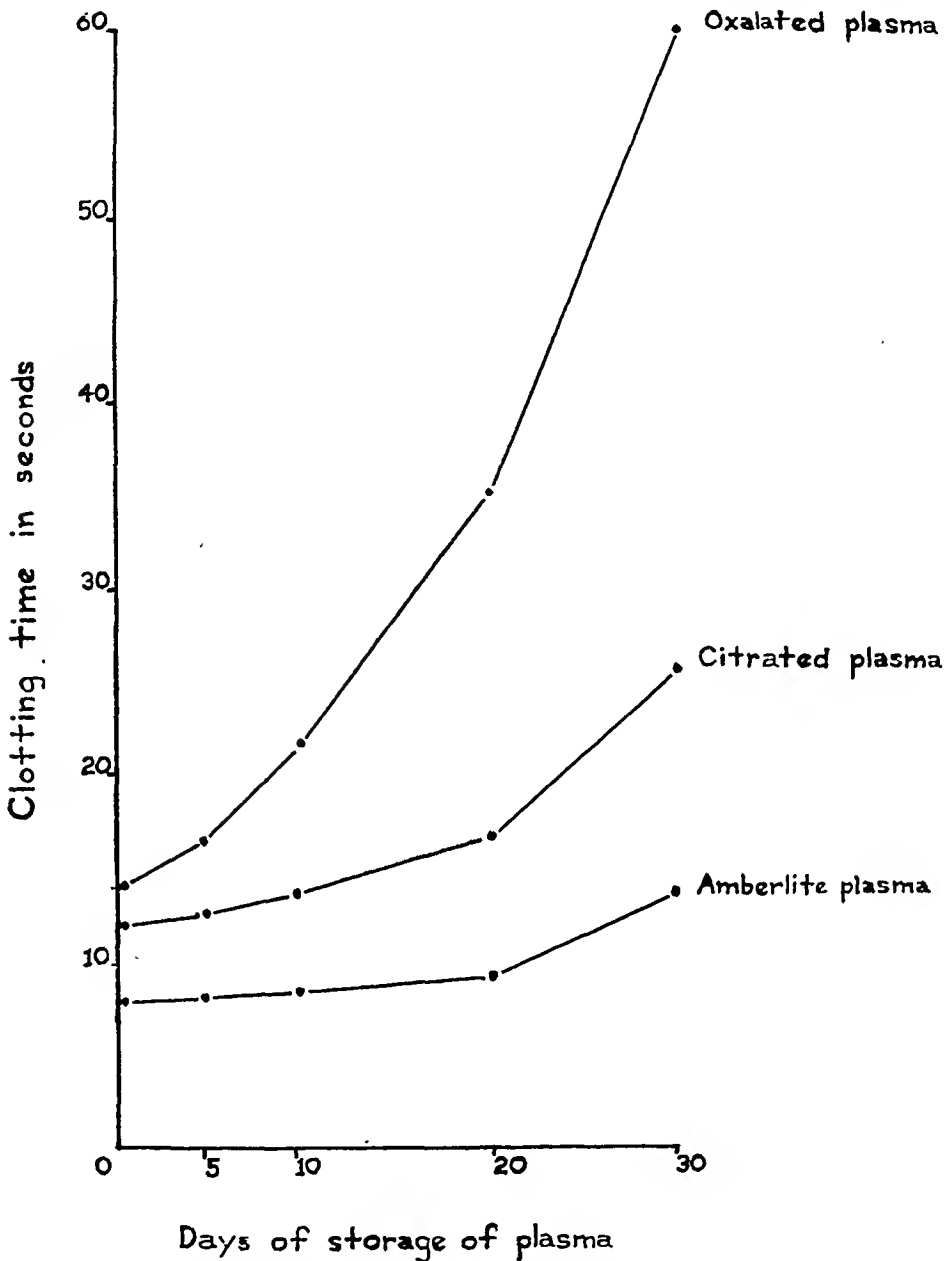


FIG. 1. Antithrombin activity of fresh and stored human oxalated, citrated and "amberlite" plasma. Period of incubation, one minute. Results of a typical experiment: equal volumes of plasma under experiment and "full strength" thrombin were incubated at 37 C. After one minute of incubation, 0.1 cc. of each mixture, separated from the clot, was added to 0.2 cc. of normal oxalated homologous plasma, and the clotting time was recorded.

prothrombin activity, and in dog or rabbit plasma in which this loss is minimal and slow. The relationship is further confirmed by the finding that plasma decalcified with 0.2 M sodium citrate shows an increase of antithrombin activity even greater than that occurring in oxalated plasma.

The extent and rate with which ionized calcium is removed from the blood are known to influence the loss of the "labile factor" of prothrombin in human blood,⁸ which, as already pointed out, is greater in oxalated than in citrated and "Amberlite" plasma. They probably also determine the different increase in antithrombin activity of stored plasma. The mechanism of this action requires further investigation.

SUMMARY

An increase of antithrombin activity occurs in plasma during storage which is usually much greater in oxalated than in citrated or "Amberlite" plasmas. The extent and speed at which calcium is removed from blood by the different decalcifying agents apparently influence the increase of antithrombin activity of stored plasma.

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AZOOSPERMIA AND ASPERMIA*

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No matter what text book one consults, the chapter on semen examination is rather brief and incomplete. Erroneous statements appear even in the latest editions. Such terms as "azoospermia" and "aspermia" are usually confused (Table 1) and are often misleadingly called "non-obstructive" and "obstructive azoospermia". Azoospermia and aspermia are two distinct entities differentiated by cytodiagnosis⁵ of the ejaculate. Testicular biopsies aid in the differentiation of alterations in azoospermia and form the basis for treatment in aspermia.

TABLE 1
DISTINCTION BETWEEN AZOOSPERMIA AND ASPERMIA

AZOOSPERMIA
No spermatozoa
CYTOSPERMIA
Testicular elements present in semen
Testicular biopsy always subnormal (hypoplasia or atrophy)
Misleadingly called "non-obstructive azoospermia"
ASPERMIA
No semen
No testicular cells, no spermatozoa
ACYTO-AZOOSPERMIA
No testicular elements in ejaculate
Testicular biopsy usually normal
Falsely called "obstructive azoospermia"

Azoospermia indicates that the semen lacks spermatozoa, but early cells of the spermatogenic series are invariably present. Such cells usually are detected in direct smears and always are found in stained films of centrifuged material. The term "cytospermia" is appropriate for such semen. In aspermia, on the other hand, the ejaculate is devoid of all testicular elements, consists of secretions of the accessory glands only and should not be called semen. The term "acytoazoospermia" could be used for such an ejaculate.

In azoospermia, the microscopic picture of the semen (spermio cytogram) reflects the quality of the germ tissue. The spermatocytes or the spermatogonia present the end state of spermatogenesis. Azoospermia occurs with testicular hypoplasia as in cryptorchidism or late descent of the testicle; atrophy as in endocrinopathies, constitutional factors, circulatory disturbances, or mechanical

* Presented at the Twenty-Sixth Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 28, 1947. Received for publication, October 28, 1947.

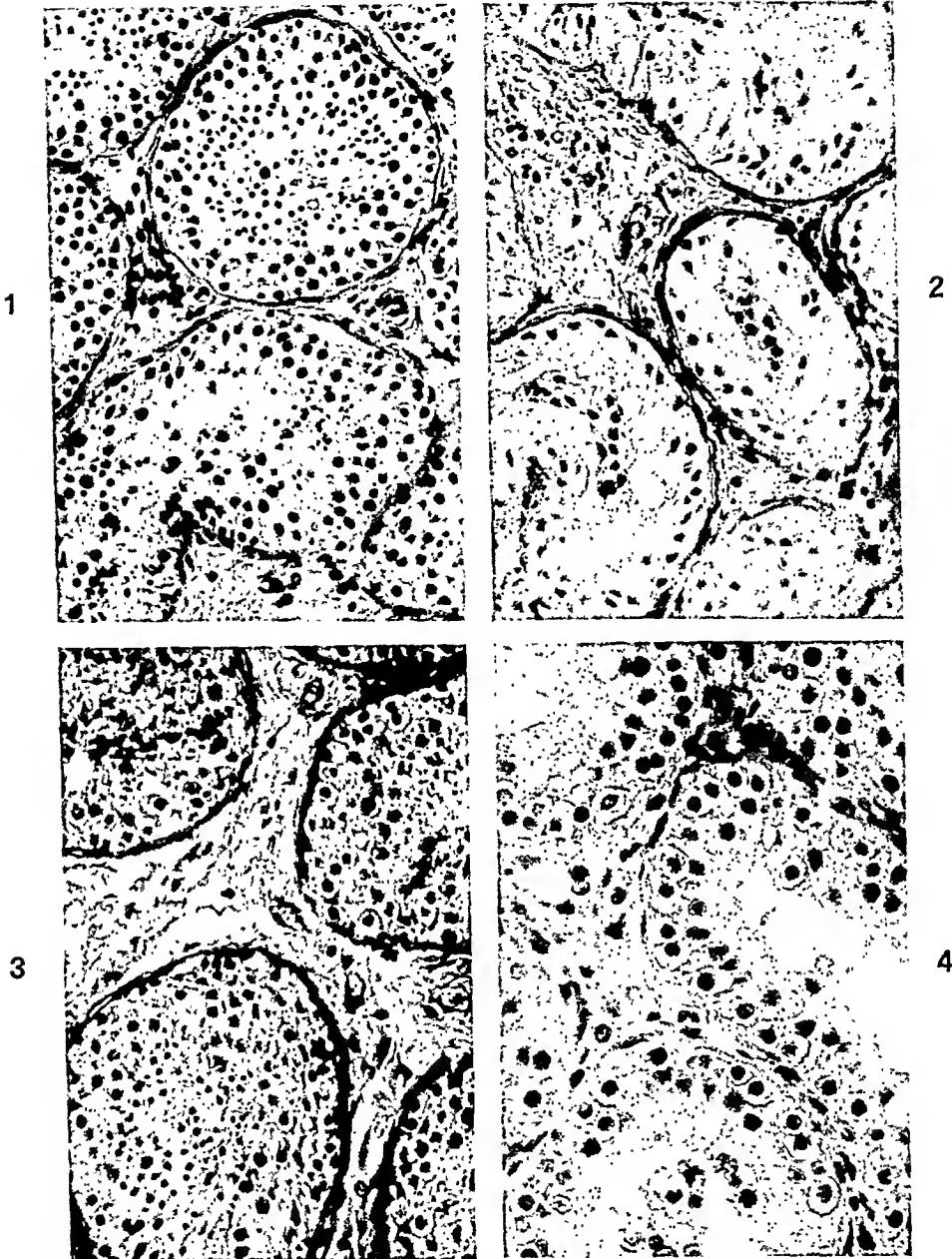
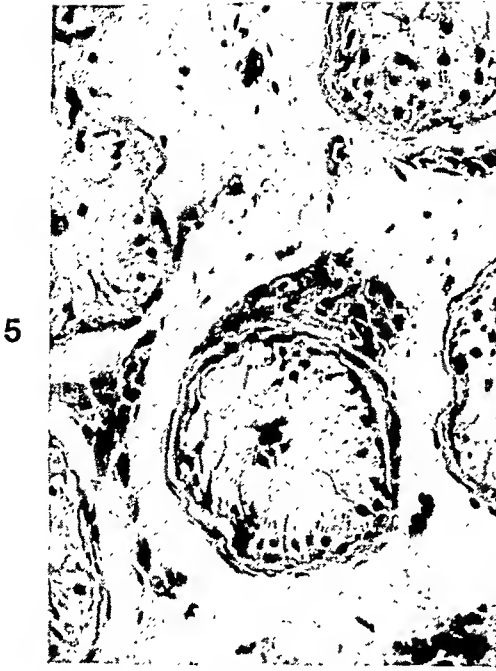


FIG. 1. Normal testicle. Testicles were of normal size. There was bilateral postgonorrhoeic epididymal obstruction. Ejaculate showed aspermia. $\times 800$.

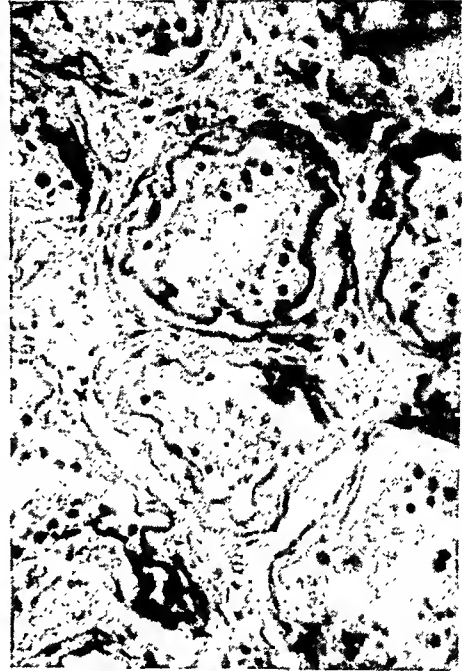
FIG. 2. Testicular hypoplasia. The right testicle had not descended; the left testicle was one-third normal size and had descended late. Ejaculate showed azoospermia. $\times 800$.

FIG. 3. Testicular atrophy, grade 1. The testicles were of normal size. The cause of the atrophy was undetermined. Ejaculate showed oligospermia. $\times 800$.

FIG. 4. Testicular atrophy, grade 2, with maturation arrest. The testicles were two-thirds normal size. The autopsy revealed a neuroblastoma of the pituitary gland. Ejaculate showed azoospermia. $\times 800$.



5



6



7



8

FIG. 5. Testicular atrophy, grade 3, with interstitial edema. The testicles were of normal size. A possible cause of the atrophy was chronic osteomyelitis. Ejaculate showed asthenospermia. $\times 800$.

FIG. 6. Testicular atrophy, grade 3, with peritubular fibrosis. The testicles were of normal size. The cause of the atrophy was undetermined. Ejaculate showed azospermia. $\times 800$.

FIG. 7. Testicular atrophy, grade 3, with hyperplasia of interstitial cells. The testicles were slightly smaller than normal. The cause of the atrophy was undetermined. Ejaculate showed azospermia. $\times 800$.

FIG. 8. Testicular atrophy, grade 3, with sclerotization of interstitial tissue. The cause of the atrophy was undetermined. (The patient was a chronic alcoholic.) The testicles were of normal size. Ejaculate showed asthenospermia. $\times 800$.

interference with spermatogenesis; and degeneration.⁶ Testicular biopsies aid in the differentiation of these changes. Hypoplasia is characterized by the presence of separated, isolated tubules which show a maturation arrest resulting from failure of development of the third zone of the germ tissue, less frequently by central collections of Sertoli's cells. Atrophy is characterized by edema and vacuolization of spermatids and even younger cells, and by decrease in the height of the germ tissue layers. Accordingly, in the presence of a single layer of cells, there are various degrees of depression of function up to cessation of spermatogenesis. At the present time, differentiation of the many causes of

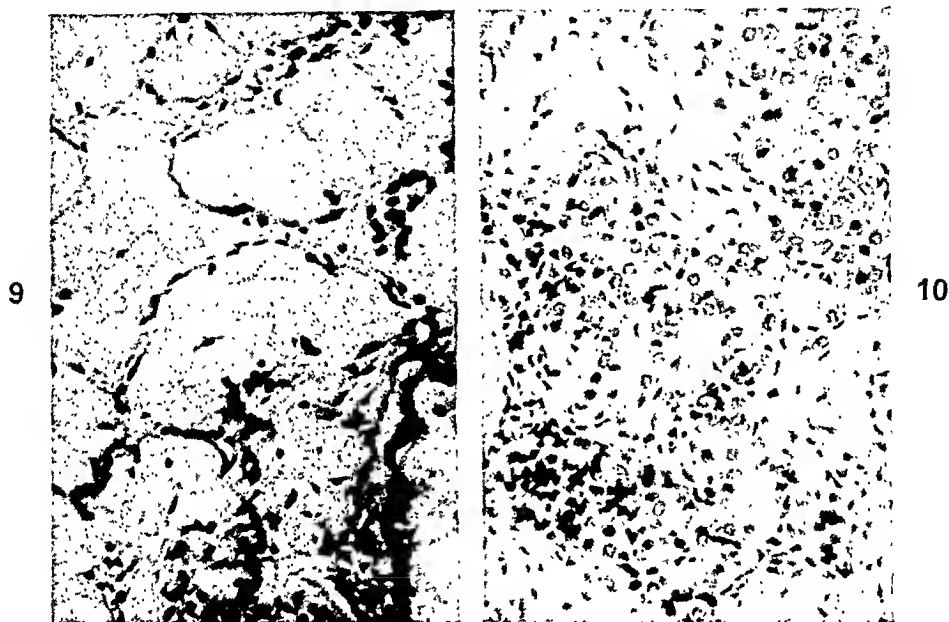


FIG. 9. Testicular degeneration with diffuse hyalinization. Testicles were of normal size. The cause of the degeneration was attributable to pulmonary and bilateral epididymal tuberculosis. Ejaculate showed aspermia. $\times 800$.

FIG. 10. Testicular degeneration with hyalinization and diffuse fibrosis. The testicles were about one-fourth the normal size. Cause of the degeneration was hypernephroma. Ejaculate showed azoospermia. $\times 800$.

these alterations cannot be based on results of testicular biopsies. The concept that interstitial cell hyperplasia characterizes an endocrine disturbance is not generally valid. Degeneration, that is, hyalinization of the tubules, is a secondary process and follows complete atrophy. The term "degeneration" is frequently used where "atrophy" is meant. In all these states there is shedding of germ cells.

It is important to perform biopsies until sufficient knowledge is accumulated to differentiate between testicular atrophy, based on certain endocrinopathies, and atrophy due to constitutional disorders, generalized infections or intoxications. Cryptorchidism can be repaired regardless of whether it is complete or not. Treatment of testicular hypoplasia with androgens is successful only

when accompanied by external hypogenitalism.² The treatment of other conditions causing azoospermia is not successful with the exception of those which are caused by local circulatory disturbances or are due to local pressure.

Aspermia is due to bilateral absence of part or all of the internal sex organs, or may be due to developmental or acquired obstruction of the seminal passages.

TABLE 2
DEVELOPMENTAL ANOMALIES WHICH HAVE A BEARING ON FERTILITY OF THE MALE

BILATERAL ANOMALIES	FINDINGS IN EJACULATE
A. <i>Testicular</i>	
Anorchidia	Aspermia
Cryptorchidism	
total	Azoospermia to normospermia*
partial	Azoospermia to normospermia*
Late descensus	
complete	Azoospermia to normospermia*
incomplete	Azoospermia to normospermia*
with epididymis	Azoospermia to normospermia*
with head only	Aspermia†‡
without epididymis	Aspermia†
Hypoplasia	
with rests of epididymis	Aspermia†
without rests	Aspermia
B. <i>Epididymis</i>	
Anepididymis	Aspermia
Autonomous descensus	Aspermia†
C. <i>Lower tract</i>	
Absence of ampulla	Aspermia
Absence of vas	Aspermia
Hypoplasia of vas	Aspermia to normospermia*
D. <i>Combinations</i>	Aspermia to normospermia*

* The findings depend on the age at descensus. The probability of events increases in the following order: normospermia, oligospermia, hypospermia, asthenospermia and azoospermia.

† Developmental obstruction.

‡ Not certain.

Postgonorrhoeic scars of the epididymis are the most common cause of aspermia and a frequent cause of male sterility. Post-traumatic scars are less common causes of aspermia while developmental anomalies are considered rare. With the present progress of therapy, complications of gonorrhea should become less and less frequent, and aspermia should become less common. Developmental anomalies^{3, 4, 8} may acquire interest, since they are not as rare as is often believed (Table 2). The biopsy of the testicle in aspermia appears normal, unless there is also one of the disorders leading to testicular alterations.

In aspermia, thorough exploration of the anatomic causes is indicated. Where aspermia is a symptom of developmental abnormalities, we have no treatment. To ascertain the patency of seminal passages, irrigation¹ of the vas deferens from the ampulla and probing⁷ of the ejaculatory ducts are attempted first. If patency cannot be established by these procedures, one can perform epididymo-vasostomy. It is imperative to ascertain the presence of normal spermatogenesis by testicular biopsies before attempting therapeutic procedures.

The occurrence of a combination of lesions should be kept in mind. Unilateral, late testicular descensus and contralateral obstruction may yield a semen which shows azoospermia while on testicular biopsy, one of the gonads may appear normal.

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A COMPARISON OF THE SENSITIVITY OF METHODS USED FOR THE DETECTION OF CARBON MONOXIDE IN BLOOD*

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Manuals of toxicology list several methods for the qualitative determination of carbon monoxide in blood,^{1-5, 7} but include few statements concerning the sensitivity of the various tests. In actual practice it sometimes happens that some of the tests are negative while others are positive.

In order to account for such apparent discrepancies, a study of the sensitivity of several common tests for carbon monoxide was made. Freshly drawn ox blood was readily available through the courtesy of the Veterinary Clinic, College of Veterinary Medicine, Ohio State University, and was used in the preliminary tests in order to conserve our supply of human blood. Blood was approximately saturated (97 per cent) with carbon monoxide generated by dropping sulfuric acid into formic acid and heating the solution. The blood containing the carbon monoxide was then diluted to a series of weaker concentration by adding untreated blood. The exact concentration of carbon monoxide in each sample was measured by the Van Slyke manometric procedure.⁶ Each of the common tests for carbon monoxide in blood was then studied and the sensitivity of the tests determined (Table 1).

These results indicate that the spectroscopic method for the qualitative determination of carbon monoxide in blood is not particularly sensitive, since in concentrations of less than 30 per cent saturation carbon monoxide cannot be detected at all. Since this method is widely used and is often selected as one of the more accurate means for the detection of carbon monoxide, it is entirely possible that the gas may have escaped detection in frequent instances. When the Hartridge Reversion Spectroscope is substituted for the ordinary biologic spectroscope, the sensitivity of the test is increased so that a concentration of 10 per cent saturation with carbon monoxide in ox blood or 8 per cent saturation with carbon monoxide in human blood can be detected. However, the Hartridge Reversion Spectroscope is an instrument manufactured only in England and is difficult to obtain at the present time.

Pyrotannic acid, the reagent used by the Bureau of Mines in its permanent standard for the rough quantitative estimation of carbon monoxide by a colorimetric comparison, detected carbon monoxide in concentrations of 8 per cent saturation in ox blood and of 4 per cent saturation in human blood. Wetzels reagent, 1 per cent tannic acid, also detected carbon monoxide in 4 per cent saturation in human blood, but in ox blood in concentrations as low as 2 per cent. Kunkel's test, in which 3 per cent tannin is used, was somewhat less

* Received for publication, January 28, 1948.

sensitive than the tests in which forms of tannic acid were used. Kunkel's test detected the gas when present in concentrations of 10 per cent saturation in human blood and in 20 per cent saturation in ox blood. None of the other tests listed in Table 1 were particularly sensitive for the qualitative estimation of carbon monoxide in human blood.

In Wetzel's test using potassium ferrocyanide, in Liebmann's test with formaldehyde and in Haldane's carmine dilution test, a carbon monoxide concentration of 20 per cent saturation was required for the detection of the gas in human blood. Rubner's test with basic lead acetate and Katayama's test with ammonium sulfide and acetic acid gave positive results only if at least 30 per cent

TABLE 1
TESTS INVESTIGATED AND THE LOWER LIMITS OF THEIR SENSITIVITIES

METHODS TESTED	LOWEST CONCENTRATIONS (PER CENT SATURATION) OF CARBON MONOXIDE AT WHICH CO WAS DETECTED	
	Ox Blood	Human Blood
	<i>per cent</i>	<i>per cent</i>
Spectroscopic examination (biologic spectroscope)	30	30
Hartridge Reversion Spectroscope	10	8
Wetzel's test (1 per cent tannic acid)	2	4
Wetzel's test (potassium ferrocyanide)	6	20
Rubner's test (basic lead acetate)	8	30
Liebmann's test (formaldehyde)	20	20
Kunkel's test (3 per cent tannin)	20	10
Katayama's test (yellow ammonium sulfide and acetic acid)	30	30
Haldane's carmine (color after dilution)	20	20
Boiling	6	30
Pyrotannic acid	8	4
Hoppe-Seyler's test (sodium hydroxide)	*	*

* Gave unsatisfactory results in all concentrations for both ox and human blood.

carbon monoxide was present in human blood. Likewise, carbon monoxide in concentrations of less than 30 per cent could not be detected by boiling human blood.

The blood samples containing the carbon monoxide were carefully kept under oil as recommended by Van Slyke. However, at the conclusion of the above tests, the protective layer of oil was removed and the samples placed in a refrigerator in contact with air. Samples were then withdrawn at various intervals up to five days in order to determine the amount of carbon monoxide likely to be lost due to contact with oxygen. However, even at the end of five days the concentration of carbon monoxide, as determined by the Van Slyke manometric procedure, remained unchanged. From these results, it may be inferred that samples of human blood to be examined for carbon monoxide need not be kept under oil.

CONCLUSIONS

Human blood must contain carbon monoxide in a saturation of at least 4 per cent before the presence of carbon monoxide can be established by qualitative laboratory tests. Pyrotannic acid and 1 per cent tannic acid (which is used in Wetzel's test) are the reagents most sensitive to the presence of carbon monoxide in human blood. The Hartridge Reversion Spectroscope will detect a concentration of 8 per cent saturation, but blood must be at least 30 per cent saturated before carbon monoxide can be detected with an ordinary biologic spectroscope.

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A RAPID METHOD FOR THE ESTIMATION OF BLOOD SUGAR*

J. KLEEBOERG, M.D.

From Medical Department A of the Rothschild-Hadassah Hospital, Jerusalem, Israel

The physician is sometimes anxious to learn the approximate level of the patient's blood sugar at once. The following method of estimation of blood sugar, despite its sacrifice of accuracy, is presented because of its advantages of rapidity and simplicity. In searching for such a method, I found that the old test of Moore was the most suitable. Although Moore's test for glucose is mentioned among the 69 different methods of examining monosaccharides described in the 1942 edition of the *American Illustrated Medical Dictionary*¹, and among the 24 tests described in Pearson and Hepburn's *Physiological and Clinical Chemistry*,³ no details are given of its use for blood and urine. Neither could I find in the Cumulative Index any publication which deals with this problem systematically, except that of Somogyi,⁵ who used Moore's test for sugar in the urine. I then applied Moore's procedure to determinations of sugar in the blood.

In this test, glucose, heated with caustic soda or potash, yields a brownish color (caramelization). To use this reaction for blood sugar, the protein-free blood filtrate is heated with potassium hydroxide, and the color is observed.

Only two chemicals are needed: (1) a 20 or 30 per cent solution of caustic potash (as in Jaffé's acetone test), and (2) a 20 per cent solution of trichloroacetic acid. The apparatus required for the test include an ordinary funnel, filtering paper, test tubes, a flame and pipets or glass syringe for measuring the fluids.

METHOD

Approximately 4 cc. of venous blood is run into a test tube containing an equal quantity of trichloroacetic acid. The mixture is then vigorously shaken, allowed to stand a few minutes, and after filtration, should yield a minimum of 3 cc. of filtrate. Any surplus of the same trichloroacetic filtrate could be used, if necessary, for the following tests on serum: indican, urea and xanthoprotein. Approximately 1.5 cc. of a 20 per cent solution of caustic potash (KOH) or 1 cc. of a 30 per cent solution is then added and the mixture boiled for one and one-half or two minutes, after which it assumes a yellow color, owing to caramelization. A glass pebble should be added or the test tube should be shaken during boiling. If a water bath is used to examine several test tubes simultaneously, the heating time should be increased to five minutes. Prolonged boiling, however, must be avoided as it causes the color to fade. (If the testing is delayed for some time after obtaining the blood specimen, a small amount of sodium fluoride may be added to the blood to serve as an anticoagulant and antiglycolytic agent.) A light yellow or a light lemon yellow color means a blood sugar level within normal limits; a distinct yellow color means an increased blood sugar level; and an orange color means a very high blood sugar value.

* Received for publication, November 25, 1947.

The control standard glucose solution should contain 130 mg. glucose in 100 cc. sterile distilled water. (A few drops of toluol may be added for preservation.) This standard solution is used in exactly the same manner as the blood filtrate: run 3 cc. of the glucose solution into a test tube, add 2 drops of the trichloroacetic acid and 1 cc. of the caustic potash, then boil simultaneously with the unknown. The color of this test tube is approximately that of a "normal" blood sugar level, ranging from 90 to 130 mg. To compare the readings, one should look at the test tubes by direct as well as by reflected light. The ordinary electric light with its yellow tinge does not interfere with proper judging of colors.

DISCUSSION

The choice of 130 mg. dextrose, instead of 100 mg. as the standard solution, was made for the following reasons: The blood filtrate of 50 patients with a normal blood sugar value between 90 and 120 mg. (determined by Folin and Wu's method²) always yielded a slightly more yellowish color than an artificial glucose solution of exactly 100 mg. per 100 ml. This slight *additional* yellow tinge of color seems to be produced by the uric acid and creatin content of the normal blood (see below). After numerous tests, it was found that a standard solution containing 130 mg. glucose per 100 ml. was more suitable for comparison. A second standard solution, containing 230 mg. glucose per 100 ml., may also be used.

The yellow color of the caramelized blood sugar remained unchanged, even in blood from patients with jaundice or under atabrine treatment. Uremia with a urea level as high as 500 mg. per 100 ml. did not alter the results, and a uric acid level of 8 or 9 mg. had no influence on the color. Concentrations greater than 9 mg. were not tested. In tests carried out on the influence of high acetone concentrations in the blood, acetone levels up to 3 mg. per 100 ml. did not affect the color, but higher concentrations rendered the yellow or orange color slightly darker.

RESULTS

This method of blood sugar estimations has been used for some time in the hospital departments and in private practice and was found to be useful in three patients in coma.

In one patient with an apoplectic coma, the urine was normal, and the blood filtrate with Moore's test showed only a slightly yellow color, indicating a normal level of blood sugar. Therefore, diabetic coma could be excluded, and the subsequent course of the disease confirmed the correctness of the diagnosis.

The second patient was a girl in her twenties, suffering from general intestinal sarcomatosis, who suddenly fell into a state of coma. There was a strong acetone reaction in the urine, and the question was whether pancreatic diabetic involvement by metastasis had to be considered and insulin therapy instituted. The "caramelization" test gave a normal blood sugar reaction and, therefore, no insulin was given. The Somogyi blood sugar analysis was made at the same time, and two hours later revealed a blood sugar value of 100 mg.

The third patient had been known to be diabetic and was admitted to the hospital one night in a state of impending coma. The urine contained sugar, acetone and diacetic acid. No insulin had been given for several days prior to admission. Fifty units of insulin were given, and blood was taken immediately for the "caramelization test". This was later

repeated at two hour intervals. The first tube with its deeply yellowish orange color was kept for comparison, while in the following tests the color grew gradually lighter, serving as a guide for the further amounts of insulin to be administered throughout the night.

Here lies another advantage of the method. Once caramelization is completed and the yellow color has formed, it does not change for ten or twelve hours. This is an important advantage for the physician in regard to those diabetics who have to be shifted from regular insulin to protamine or globin-zinc insulin or similar preparations. When the first test is made in the early morning before the insulin injection, one keeps this first test tube with its stable color in the rack for comparison with those blood samples which will be taken later in the day at four or six hour intervals. By merely comparing the colors of these different tubes, the physician can immediately perceive the influence of administered protamine zinc insulin on the blood sugar throughout the day.

The degree of caramelization is related directly to the amount of sugar in the blood filtrate. A low blood sugar will produce only a weak caramel reaction. Thus, hypoglycemia can be recognized easily by the very light yellow color of the blood filtrate. In extreme hypoglycemia the fluid in the test tube may remain colorless. The caramelization test (Moore) has one disadvantage in that it requires a venous puncture. All attempts to develop a satisfactory micro-method have failed. Thus far, I have not been able to prepare preservable solutions of potassium bichromate or of iodine-alcohol solutions (as described by Somogyi for his urine test). A small set of such preservable color solutions in tubes might be useful, but it is by no means necessary. On the other hand, it is an advantage of the method that the practitioner or hospital assistant is not dependent on special apparatus to perform this quick test.

The method just described cannot and will not replace the exact blood sugar micro-methods. The use of this caramelization test should be limited to emergency cases or for obtaining a quick approximate idea of a patient's condition when apparatus or special laboratory facilities are not available.

SUMMARY

A simple test, which enables the estimation of blood sugar in a few minutes without special equipment or chemicals, is described. The method is based upon the old Moore reaction. Both hyperglycemia and hypoglycemia can be determined. The test is accurate enough to follow the effect of protamine-zinc insulin.

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SELECTED ABSTRACTS

Convulsions Under General Anesthesia. J. BARCHAM AND B. H. ELIASBERG. J. Mt. Sinai Hosp., N. Y., 14: 912-917, 1948.

Five cases of convulsions occurring under general anesthesia, 1 with fatal termination, are reported. The junior author previously had not seen a single case in 50,000 anesthetics personally administered, but the average is said to be 1 in 6000 cases. Mortality figures vary from 18 per cent to as high as 50 per cent. The etiology of the convulsive seizure is not known, but the condition occurs more often in young people and in persons with evidence of sepsis and toxemia. The authors recommend the use of a non-inhalation agent under such circumstances; if that is not possible, an anesthetic other than ether should be employed.

This reviewer encountered 2 fatal instances in operations of appendectomy for acute appendicitis in a single week in one hospital during a summer heat spell. Both patients (a girl of 13, and a young man of 20 years) were apparently relatively dehydrated, received ether and were given oxygen and stimulants instead of the obviously indicated depressants upon the onset of convulsions.

Fort Wayne, Indiana

S. M. RABSON

Familial Idio-pathic Methaemoglobinaemia. Q. H. GIBSON AND D. C. HARRISON. Lancet, 2: 941, 1947.

Before attempting to indict an industrial hazard as a cause of hemoglobinemia, the familial idiopathic variety of methemoglobinemia should be included in the differential diagnosis. Gibson and Harrison record a family of 9 children, in which 5 siblings (2 females and 3 males) had the disease. The blood oxygen dissociation curve of 1 patient showed a shift to the left. The authors believe the condition is not one of a bone marrow defect but of a disturbance in the equilibrium between hemoglobin (ferrous) and methemoglobin (ferric), with both substances present in all cells. Methemoglobin is always being formed in normal persons, but is considered reduced by the triose phosphoric and lactic enzymes of the erythrocytes (Gibson). Deficiency of these enzyme systems causes elevation of the methemoglobin in the blood. The efficacy of methylene blue probably depends on its catalytic action, while ascorbic acid is effective by direct reaction with methemoglobin. Proper ascorbic acid therapy (0.3-0.4 grams daily) will cause the methemoglobin level to be about 1 gram per 100 ml. blood, but the dye is also occasionally used (0.3 grams by mouth) if cyanosis tends to return.

S. M. RABSON

Effect of Acetone and Alcohol Fixation and Paraffin Embedding on Activity of Acid and Alkaline Phosphatases in Rat Tissues. ROBERT O. STAFFORD AND WILLIAM B. ATKINSON. Science, 107: 279-281, 1948.

Acid phosphatase is more difficult to demonstrate histochemically than alkaline phosphatase. Acetone fixation gives better preservation of the acid phosphatase activity than does alcohol fixation.

Acid and alkaline phosphatase activity was measured in tissue freshly removed from the animals, as well as tissues which were fixed for varying times in acetone and alcohol, and tissues which were fixed, embedded in paraffin and sectioned and deparaffinized. The method of Huggins and Talalay was used to determine the enzyme activity. It was found that acid phosphatase was inactivated by acetone and alcohol fixation to a far greater extent than alkaline phosphatase. Alcohol fixative preserves a somewhat greater amount of alkaline phosphatase activity than does acetone. Paraffin embedded tissues were found to have approximately 5 per cent of acid phosphatase activity left, and from 20 to 30 per cent of alkaline phosphatase activity remaining.

Cleveland

BEN FISHER

Studies in Protein Metabolism with Compounds Labeled with Radioactive Carbon. I. Metabolism of dl-Tyrosine in the Normal and Tumor-Bearing Rat. T. WINNICK, F. FRIEDBERG AND D. M. GREENBERG. *J. Biol. Chem.*, **173**: 189-197, 1948.

The administration to rats of dl-tyrosine, tagged with the radioactive C¹⁴ isotope, furnished the tool for comparative study of protein metabolism in normal and tumor-bearing animals. The relative concentration of the amino acid in the various organs followed the same pattern in normal and lymphosarcomatous animals. The tumors, however, surpassed normal organs regarding the incorporation of tyrosine: they contained 25 to 33 per cent of the total C¹⁴ taken up by the body protein, although the tumors represented only 9 to 11 per cent of body weight. This study confirms the high activity of protein synthesis which is manifested by malignant tumors and which obviously is connected with their growth potential.

Chicago

KURT STERN

In-Vivo Staining and Retardation of Tumors in Mice by Acridine Compounds. M. R. LEWIS AND P. P. GOLAND. *Am. J. M. Sc.*, **215**: 282-289, 1948.

In this large-scale study 331 acridine compounds were tested as to their effect on transplanted sarcomas and spontaneous mammary carcinomas of mice. The dyestuffs were administered orally. It was found that 204 compounds produced vital staining of the tumors, and most of these dyes depressed the rate of tumor growth. Sixteen acridine derivatives retarded tumor growth considerably (treated tumors being from 1/20 to 1/40 of the size of untreated ones), but no prevention of tumor growth or complete regressions were observed. The tumor-retarding effect of the dyes was thought to be due to slowing of the mitotic rate of the tumor cells.

KURT STERN

Deposition of Liver Glycogen in Normal Mice and in Mice Bearing Sarcoma 180. N. F. YOUNG, C. J. KENSLER, LOUISE SEKI AND F. HOMBURGER. *Proc. Soc. Exper. Biol. and Med.*, **66**: 322-323, 1947.

The hepatic glycogen storage following intraperitoneal glucose injection was determined in normal mice and in mice bearing implants of sarcoma 180. The fasting levels of hepatic glycogen were found to differ only insignificantly in tumor-free and tumor-bearing animals.

However, tumor-bearing animals were able to convert less than 12 per cent of the injected glucose into liver glycogen, in contrast to a conversion of more than 20 per cent accomplished by normal mice. These experimental results are in line with clinical findings obtained by the Memorial Hospital group in human gastric cancer; these patients, too, exhibited an impaired ability of storing hepatic glycogen.

The mechanism of this metabolic defect, observed in both man and animal in connection with cancer, requires further investigation. It may be due to hepatic dysfunction resulting from the tumor process; it is also conceivable that it is tied up with the carbohydrate metabolism peculiar to malignant tumors.

KURT STERN

BOOK REVIEWS

The Biology of Melanomas. Special Pubs. Volume IV. By MYRON GORDON, GLENN H. ALGIRE, S. WILLIAM BECKER, HAROLD F. BLUM, LIANE R. BARUCH, E. A. SIEREMETIEVA-BRUNST, V. V. BRUNST, DEAN BURK, GLADYS CAMERON, GRAHAM PHILLIPS DUSHANE, FRANK H. J. FIGGE, CLARA E. FISCHER, DENIS L. FOX, SAMUEL A. GOLDBERG, C. G. GRAND, JESSE P. GREENSTEIN, JAMES B. HAMILTON, R. G. HARRISON, MARIE L. HESSELBACH, M. J. KOPAC, FRANCES Y. LEGALLAIS, MICHAEL LEVINE, ELEANOR J. MACDONALD, MADGE THURLOW MACKLIN, HOWARD S. MASON, P. MASSON, J. M. ODORNE, GEORGE T. PACK, ELIZABETH S. RUSSELL, W. L. RUSSELL, LEONELL C. STRONG, KANEMATSU SUGIURA, HELENOR CAMPBELL WILDER and B. H. WILLIER. 466 pp., 107 plates, 28 tables, 23 figs. \$5.00. \$4.00 to members of the New York Academy of Sciences. New York: The New York Academy of Sciences, 1948.

This book consists of 26 individual articles on all phases of the biology of melanomas, including clinical, developmental, cytologic, genetic and endocrinologic. These papers were presented before a conference sponsored by the Section of Biology of the New York Academy of Sciences, and they contain information relative to all of these fields integrated under the subject of pigment cell biology. The papers vary in length from 4 to 53 pages and are well illustrated. The photomicrographs, particularly in the chapters by Masson and Becker, are excellent. There is also a selective bibliography and a good index. This monograph should prove of special interest to the pathologist interested in dermatology and neoplasms. Because of grants in aid made to the New York Academy of Sciences, the price of the book has been kept at a very reasonable figure.

It may perhaps be considered that some chapters are not of practical value. However, in the understanding of melanin pigmentation, moles and melanocarcinoma, all of the material presented is related to some degree. The fundamental studies of P. Masson under the title of "Pigment Cells in Man" and of S. William Becker entitled, "Dermatological Investigation of Melanin Pigmentation", should be of interest to all pathologists. These men discuss in detail melanin pigmentation and the histogenesis of melanocarcinomas, and both give the uses and technique of the dopa test. Becker also discusses other pigmented skin lesions. Eleanor J. MacDonald in "Malignant Melanoma in Connecticut" points out that registration of cancer has proved its value. She cites the incidence and curability of malignant melanoma in that state and reports malignant melanoma in 6 patients under the age of 15 years.

The following chapters are mentioned to show the scope of the book: "Relationship of Pigment Cell Clusters in the Iris to Malignant Melanoma of the Uveal Tract", by Helenor Campbell Wilder; "The Genetic Aspects of Pigment Cell Growth in Man", by Madge Thurlow Macklin; "The Growth and Vascularization of Transplanted Mouse Melanomas", by Glenn H. Algire and Frances Y. Legallais; "Tissue Culture Studies of Pigmented Melanomas in Fish, Mouse and Man", by C. G. Grand and Gladys Cameron; "The Influence of the Endocrine Status Upon Pigmentation in Man and Mammals", by James B. Hamilton; "Induction of Melanotic Tumors and Pigmented Hair Changes in Mice by Methyleholanthrene", by Leonell C. Strong; "The Chemistry of Melanomas", by Jesse P. Greenstein; and "Light and Melanin Pigment in Human Skin", by Harold F. Blum.

It would have been helpful to the pathologist to have had an article or articles detailing the early changes which would allow one to call a mole malignant, as well as a detailed description of the histologic changes in melanocarcinoma. There is a clinical paper by George T. Pack which stresses surgical treatment.

St. Louis

LAUREN V. ACKERMAN

Blood Derivatives and Substitutes. Preparation, Storage, Administration and Clinical Results Including a Discussion of Shock: Etiology, Physiology, Pathology and Management. By CHARLES STANLEY WHITE, M.D., Sc.D., Former Professor of Surgery, George Wash-

ington University School of Medicine, and Chief of Surgery, Doctors Hospital, and JACOB JOSEPH WEINSTEIN, B.S., M.D., Associate in Surgery, School of Medicine, George Washington University Hospital, Washington, D. C. 484 pp., 190 figs. \$7.50. Baltimore: The Williams & Wilkins Company, 1947.

The therapeutic use of blood plasma and of other blood derivatives and substitutes has been growing so rapidly that few of us have been able to keep pace with the avalanche of papers distributed in periodicals. The authors of this book have attempted to present a review of the whole subject. Both men are eminently qualified, having taken an active part in the development of this relatively new branch of therapeutics.

The 15 chapters deal with history of transfusions and blood banks, chemistry and physiology of plasma, preparation and storage of liquid citrated plasma and of dried plasma, administration, plasma fractions, by-products and substitutes, serum, blood preservation, transportation, the universal donor, blood plasma bank, shock, clinical results with plasma and reactions.

Every one of the subjects is of interest and of great practical importance to the clinical pathologist. There is an abundance of technical details clearly and exhaustively presented. The illustrations are well selected and add to the understanding of various technics. Well-chosen lists of references follow each chapter.

This book is a must for every one interested in blood and plasma transfusions. It is the most complete and up-to-date presentation of an expanding field where clinical medicine, surgery and clinical pathology meet and have much to offer to each other.

Chicago

I. DAVIDSOHN

Laboratory Technique in Biology and Medicine. By E. V. COWDRY, Professor of Anatomy, Washington University, and Director of Research, The Barnard Free Skin and Cancer Hospital, St. Louis. 269 pp. \$4.00. Baltimore: The Williams & Wilkins Co., 1948.

This second edition of Cowdry's book retains the general organization of the first edition, but contains a large amount of added important new material, especially in the fields of histochemistry and radioactive technics. Practically all the recent methods are adequately covered; many of the original entries have been completely rewritten and considerably expanded to include modern developments.

It may be questioned whether some of the additions (e.g., the detailed historical description of some obsolete dyes such as fustic, Tyrian purple and woad) serve any useful purpose. A few disturbing misprints (decolorization of acid-fast stain with sodium sulphate, p. 71; Mg instead of Hg in the reaction for tyrosine, p. 255) and errors (formol-Zenker the preferred fixative for acid-fast stain; chromaffin reaction can be performed after fixation in dichromate-free media; phenolphthalein contains sulfuric acid) have been taken over unchanged from the first edition.

On the whole, however, it may be safely asserted that this book contains incomparably more useful and practical information for the biologist than any other book of similar size.

Chicago

GEORGE GOMORI

Stereoscopic Atlas of Neuroanatomy. By H. S. RUBINSTEIN, M.D., Ph.D., Director of the Alfred Ullman Laboratory for Neuro-psychiatric Research, Sinai Hospital, Baltimore, Maryland; and C. L. DAVIS, M.D., Professor of Anatomy, School of Medicine, University of Maryland. 19 pp., 43 plates. \$10.00. New York: Grune and Stratton, 1947.

This addition to the long lists of atlases which have appeared in the last few years purports to be an aid in orientation in courses in neuroanatomy and a useful review tool for those preparing for "board" examinations. The reviewer has grave doubts that it will be of any great value in accomplishing either of these tasks. If the undergraduate student performs the dissections as set forth in the laboratory outline, these pictures are superfluous; and if he does not, all the three-dimensional pictures in the world will not give him the concepts he should have. The "board" candidate needs experience in brain dissection

as much as, if not more than, the undergraduate student. This seems to the reviewer to be another situation where the elaborate mechanisms of "visual education" do not accomplish what they set out to. Even in the year 1948 there remains some pain attached to the education of a physician.

The reader is quite correct in concluding that the reviewer is more than somewhat allergic to the general idea of atlases. However, he is willing to admit that there are good and bad ones. The present effort is clearly in the latter category. The diagrams accompanying the stereograms are not good; they lack the clarity that diagrams should have and tend to confuse with their multiplicity of labels. Most of the photographs are amateurish in that they suffer from bad lighting, lack of depth and lack of detail. Some of the specimens appear to have become dried and to have assumed the brownish coloration characteristic of nervous tissue. These patches appear dark gray to black and lend nothing to esthetic values. It is regrettable that many good dissections of the brain should have been spoiled by amateurish photography. It is to be hoped that the dissections have been placed in suitable containers and made available to students at the University of Maryland.

Detroit

GORDON H. SCOTT

The Sulfonamides and Allied Compounds. By ELMORE H. NORTHEY, Ph.D., Administrative Director, Stamford Research Laboratories, American Cyanamid Co., Stamford-Conn. 660 pp., 323 tables. \$15.00. New York: Reinhold Publishing Corp., 1948.

This comprehensive monograph covers the chemistry of over 5000 sulfonamide compounds. Of particular interest to the clinical pathologist is the chapter on the pharmacology of sulfonamide and sulfone drugs which includes a discussion of methods of assay in body fluids, toxicity studies, absorption and excretion studies and invaluable information on the distribution of these compounds in the tissues of the host. Of interest to the therapist is the maxim: "A drug which does not distribute itself in all the tissues of the body may possess *in vitro* activity and yet be of little value therapeutically because it fails to reach the site of the infection." With this in mind Dr. Northey exhaustively discusses tissue distribution of the sulfonamides.

Outstanding in the text is a discussion of the theories of the mechanism of action of sulfonamide drugs with rather detailed treatment of sulfonamide antagonists and potentiators. In this respect the pathologist will find valuable information on the detection of resistant organisms, the effects of sulfonamide drugs on enzymes and the effects of sulfonamides on various body tissues as well as on the bacterial invaders. The chapter on clinical evaluation of these drugs is both orderly and comprehensive.

This book handles a difficult subject with clarity and in an objective style. It is not only an excellent reference in itself but contains a bibliography of over 2500 other references.

Detroit

MARK DALE

OBITUARIES



PHILIP HILLKOWITZ

Philip Hillkowitz, charter member and first president of the American Society of Clinical Pathologists, died January 30, 1948, in Mercy Hospital, Denver, Colorado, following a cerebral hemorrhage. Dr. Hillkowitz was born August 30, 1873, in Salant, Lithuania, and came to the United States with his parents at the age of 11. After graduation from the Medical College of Ohio in Cincinnati in 1897, he established a practice in Denver. In 1901 Dr. Hillkowitz was elected president of the Jewish Consumptive Relief Society, a position which he held until his death. In order to concentrate on the program of the Relief Society and to devote himself to clinical pathology, he discontinued his general practice in 1910. During World War I, he served as a captain in the medical corps. In 1922 he was elected the first president of the American Society of Clinical Pathologists, and for twelve years he served as chairman of the Board of Registry of Medical Technologists. The author of many articles, his clinical achievements are written in the publication, *American Men of Science*. Dr. Hillkowitz was a past-president of the Denver County Medical Society, a member of the American Medical Association and was director of the library of the Denver Medical Society. He was a professor of pathology in the University of Colorado for several years and served as director of laboratories at Beth Israel Hospital, Mercy Hospital and St. Anthony's Hospital in Denver, and St. Joseph's Hospital in Al-

liance, Nebraska. He is survived by his wife, Minnie; a daughter, Mrs. R. B. Armstrong of Folsom, California; and a sister, Mrs. Anna Bresler of San Diego, California.

FREDERIC E. SONDERN

Frederic E. Sondern was born March 30, 1867, in Stuttgart, Germany and died October 10, 1947. His early education was obtained in the public schools of New York City, where he was graduated from high school at the age of 16. He then spent the next three years at the Universities of Heidelberg and Tübingen. He returned to the United States in 1886, and enrolled in the college of Physicians and Surgeons of Columbia University, graduating in 1889. He interned at Lenox Hill Hospital, New York, where he met Dr. Abraham Jacobi with whom he became associated for the following six years. In 1898 Dr. Sondern established the first private clinical laboratory in New York City and from then on devoted himself exclusively to this specialty. He became Professor of Clinical Pathology at the New York Post Graduate Medical School and Hospital, Director of the Clinical Laboratories of the New York Lying-In Hospital, Clinical Pathologist at Roosevelt Hospital and to the Laboratory of the Out Patient Department at Bellevue Hospital. He was a charter member of the American Society of Clinical Pathologists and an active fellow up to 1937 when he was elected to honorary membership. He was President of the Society in 1925-26, served as Counselor for the State of New York, on the Executive Committee and on various other committees. He was a member of the American Medical Association, New York County Medical Society (past president), New York State Medical Society (past president), New York Academy of Medicine, International Society of Urologists, American Association of Pathologists and Bacteriologists, American Society of Immunologists, New York Pathological Society, New York Clinical Society, German Medical Society of the City of New York, and for many years, served as President of the Board of Trustees of the New York Post Graduate Medical School and Hospital, and Trustee of the Good Samaritan Dispensary.

CLEMENT COLEMAN FENTON

Clement Coleman Fenton was born in New York City in 1892, and died May 28, 1947, of a coronary occlusion. Dr. Fenton received his A.B. degree from Columbia University in 1915, his M.S. degree from West Virginia University in 1922, and his M.D. degree from Cornell Medical School in 1925. He interned at Altoona Hospital, Altoona, Pennsylvania, and Fordham Hospital, New York City. He took post graduate work at Western Reserve University School of Medicine, the University of Minnesota, and in Berlin and Vienna. He was Professor of Pathology and Clinical Pathology at West Virginia University School of Medicine from 1926, and was consultant in pathology to several hospitals in West Virginia. In October 1945, he organized the Association of Pathologists of West Virginia and served as president up to the time of his death. He was a member of the following organizations: Monongalia County Medical Society (past president), West Virginia State Medical Society, American Medical Association, Southern Medical Association, American College of Physicians, American Association of Pathologists and Bacteriologists and the International Association of Medical Museums. He was a thirty-second degree mason, a member of the Shrine and was a Lieutenant-Commander in the United States Naval Reserve, Medical Corps. Dr. Fenton became a member of the American Society of Clinical Pathologists in 1938, and was certified in clinical pathology and pathologic anatomy by the American Board of Pathology.

GEORGE IVES

George Ives, a charter member of the American Society of Clinical Pathologists, died in St. Louis, Missouri, February 2, 1948, at the age of 64. He was born October 21, 1883, in Fort Atkinson, Wisconsin, received his A.B. degree from the University of Wisconsin in 1907 and his M.D. from Johns Hopkins University in 1911. Dr. Ives was Assistant in Physiology at the University of Wisconsin from 1907 to 1908, Assistant Professor of Bac-

teriology at St. Louis University from 1912 to 1914, Assistant in Dermatology at Washington University from 1915 to 1916 and Assistant in Medicine from 1916 to 1919. For the last thirty-three years he served as Pathologist to the Missouri Baptist Hospital, and for the last thirteen years was Pathologist to The Josephine Memorial Hospital, St. Louis. He was formerly Pathologist to St. Johns, Mullanphy and Jewish Hospitals, St. Louis. A member of the St. Louis Medical Society, Missouri State Medical Society, American Medical Association, American Public Health Association, St. Louis Clinics and St. Louis Surgical Society, he was also a Diplomate of the American Board of Pathology and was instrumental in the organization of the American Society of Clinical Pathologists. He called the first meeting of the Society in St. Louis in 1921, was its first Vice-President and later served as a member of the Board of Censors. He was a member of the Wisconsin National Guard from 1902 to 1904, University Club of St. Louis and was a 32nd Degree Scottish Rite Mason. Dr. Ivcs was the author of many scientific papers. He is survived by his wife, two daughters and two sons.

BRUCE S. WEAVER

Bruce S. Weaver died January 8, 1948, at the age of 67. He received his medical degree from the University of Michigan in 1910. After serving as pathologist in New York City, he moved to Stamford, Connecticut, where he practiced general medicine and became Bacteriologist and Pathologist at Stamford Hospital. Since the founding of St. Joseph's Hospital in Stamford in 1942, he was Pathologist and Bacteriologist there and was serving in that capacity at the time of his death. Dr. Weaver was a member of the Stamford, Fairfield County, Connecticut State Societies and American Medical Association. He was a Diplomate of the American Board of Pathology and became a member of the American Society of Clinical Pathologists in 1941.

GEORGE C. BOWER

George C. Bower, Director of Clinical Laboratories at the Marcy State Hospital, Marcy, New York, died suddenly December 1, 1947 at the age of 49. Dr. Bower was born in Blaisdell, New York, received his early education there and attended the University of Buffalo, graduating in medicine in 1922. Following his internship in the Erie County and the Deaconess Hospitals, Buffalo, he joined the staff of the Willard State Hospital, Willard, New York, and was advanced to the position of Pathologist two years later. He was appointed Pathologist at the Marcy State Hospital in 1933. Dr. Bower was a fellow of the American College of Pathology and was the author of many articles dealing with both general pathology and neuropathology. Among the professional societies to which Dr. Bower belonged were the American Medical Association, Oncida County Medical Society, Utica Academy of Medicine, New York State Society of Pathologists and the Mohawk Valley Neuropsychiatric Society. He was a diplomate of the American Board of Pathology. Dr. Bower became a member of the American Society of Clinical Pathologists November 10, 1936. He is survived by his wife and daughter.

LAURENCE COLEMAN MILSTEAD

Laurence Coleman Milstead died in Quincy, Pennsylvania, August 31, 1947, of acute myocardial failure at the age of 47. He was graduated from the Georgetown University School of Medicine, Washington, D. C., in 1924. Dr. Milstead served during World War II and was Pathologist to St. Mary's Hospital, Quincy. He was certified by the American Board of Pathology, became a member of the American Society of Clinical Pathologists in 1937, was a member of the Medical Society of the State of Pennsylvania and a fellow of the American College of Physicians.

NEWS AND NOTICES

COMMITTEE ON TUMOR TERMINOLOGY

On February 27, 1948, the Sub-committee on Oncology of the Committee on Pathology of the National Research Council met in Washington, D. C. This meeting was attended by Dr. Isabella H. Perry, Chairman of the Committee on Tumor Terminology of the American Society of Clinical Pathologists.

After a careful exploration of the plans and purposes of the two committees it was agreed that both committees have a common purpose of promoting clarity and unity in tumor terminology. This is only part of a more extensive program of the Sub-committee on Oncology.

To avoid overlapping expenditures of effort and funds, and to avoid any confusion that might arise from dual activities in a common field, Dr. Perry then recommended to the officers of the American Society of Clinical Pathologists that the objectives of the committee, clarity and unity in tumor terminology, were best promoted if for the present the Committee on Tumor Terminology restrict its activity; that the Committee and the Consultative Panel and the group of Specialist Advisors maintain their organization until their services are timely; and that the Committee on Tumor Terminology remain in communication with the Sub-committee on Oncology for mutual cooperation in attaining their common goals. The officers of the American Society of Clinical Pathologists accepted this recommendation.

According to Dr. Perry, the problems in tumor terminology lie in three great fields: (1) A heterogeneous field of known facts which may be more usefully organized by scholarship; (2) a large field of ignorance to be eroded by research; (3) the psychology of inter-group relationships in which cooperation is essential. Dr. Perry takes this opportunity to thank the members of the Committee on Tumor Terminology, the officers of the American Society of Clinical Pathologists, the representatives of the Consultative Panel and Specialist Advisors and the Sub-committee on Oncology for their perception of the problems in tumor terminology and their willingness to work on the solution of these problems.

The International Society of Hematology will hold its bi-annual meeting at the Hotel Statler in Buffalo, New York, August 23-26, 1948.

The following article from *Minnesota Medicine* (31: 81-82, January 1948) is reprinted through the courtesy of the editor of *Minnesota Medicine*. This article was prepared at the suggestion of The Committee of the Minnesota State Medical Association "to enlighten the Association on the position the clinical pathologist takes on the diagnostic laboratory services rendered by the State Board of Health Laboratory with special reference to the Rh determination. The opinions expressed, however, are the author's own."

THE CLINICAL PATHOLOGIST VERSUS THE STATE HEALTH LABORATORY

During the annual session of the Minnesota State Medical Association, held at Duluth in July, 1947, the House of Delegates passed a resolution petitioning "the State Board of Health to include in its laboratory service Rh typing in order to make this service readily available to all practicing physicians".

This action was taken apparently without having given due consideration either to the fundamental merit of the proposition, or to the sentiments of the practicing clinical pathologists who hold membership in the Association.

The clinical pathologist objects to the resolution on the following grounds:

(1) The clinical pathologist, a fellow practitioner and member of the Association, who is materially affected by this resolution, had not been consulted before it was acted upon.

The Rh determination is admittedly a laboratory procedure, and as such, it belongs in the sphere of the practice of clinical pathology.

(2) The Rh determination is not an emergency measure affecting public health, nor is it related to the control of communicable diseases. It can be carried out in the usual course of practice in any existing laboratory.

(3) The Rh determination by the state laboratory would invariably lead to blood grouping in general, as well as the Rh typing of male members of the population, since they are closely related in transfusion practice.

(4) Newly married or pregnant women may have their Rh determined at any time, not by the state laboratory as a public health or emergency measure, but by a private laboratory purely as a routine procedure.

(5) The technique of Rh determination is as simple as that of blood grouping and cross matching, since potent and reliable anti-Rh sera are now plentiful. The difficulty encountered in the past which probably prompted the resolution, was due mainly to the scarcity of reliable anti-Rh sera. This situation no longer exists, and all technicians who are trained to do blood grouping can perform the Rh test with equal skill and accuracy. The only caution suggested here would be that facilities for consultation should be made readily available in doubtful or important cases.

(6) The clinical pathologist claims that, since the Rh determination is within the sphere of his specialty, he may rightly expect a revenue from the performance of these tests for the practitioners. To deny him this right by the official action of his fellow practitioners would seem uncalled for, since there are a number of clinical laboratories throughout the state, directed or advised by qualified clinical pathologists, which are equipped to render not only the simple Rh determination but the more complicated serological procedures which may be required in the diagnosis and treatment of persons suffering from Rh incompatibility. It should be emphasized here that the clinical pathologist is fully cognizant of his obligations to the indigent and would gladly offer his services to him without cost whenever requested to do so.

DISCUSSION

The clinical pathologist is a practitioner of medicine in the same sense as any of the members of other specialties. He is entitled to the support and protection of organized medicine. His future destiny may affect the destinies of all other members of the medical profession.

Unfortunately for the professional and economic security of the clinical pathologist, the diagnostic services of the state laboratory in infectious and communicable diseases in recent years have developed to such an extent that the medical profession looks to it more and more for aid in laboratory diagnosis, not only because of the excellent service it renders, but because of the elimination of the expense which it otherwise entails. The practice is now so universally appreciated, there is a tendency on the part of the practicing physicians not only to take full advantage of the present facilities of the state laboratory, but to advocate the extension of its services to include all diagnostic tests in order to "cushion" the cost of such services to the patient, ignoring completely the place which the clinical pathologist occupies in the practice of medicine. Coupled with this unfortunate trend is a more fundamental, yet less appreciated, fact that the field of clinical pathology and laboratory medicines, by its very nature, conveniently furnishes a fertile soil for the propagation of the seed of state controlled medicine, and that, combined with the public health aspects of the medical practice in general, the state laboratory may become the spearhead of socialized medicine, which may eventually affect not only the practice of clinical pathology, but that of radiology, pediatrics, obstetrics, or any other specialty.

This situation which threatens the very existence of the clinical pathologist as a medical specialist, and which, indeed, denies the principle of free private enterprise to a group of practitioners, is probably an important factor in the present scarcity of clinical patholo-

gists. Young graduates are hesitant and cannot easily be persuaded to specialize in clinical pathology, since they realize full well that the future of the specialty seems uncertain and uninviting.

This means that the smaller hospital and communities will continue to be deprived of adequate clinical laboratory service except through the facilities of remote metropolitan laboratories, or through the services of the state laboratory.

In order to remedy this unsatisfactory situation, and to obtain adequate and on the spot laboratory service for rural hospitals and practitioners, and in order to uphold the principle of private enterprise for all, the clinical pathologist would propose to interest young medical graduates to enter into the specialty by assuring them of a productive field from which they may derive an attractive and satisfying practice, without the fear of "competition" from the state laboratory. To that end, he would suggest the establishment of a number of local community health centers in various parts of the state, each including a well-equipped hospital with adequate laboratory service under the direction of a trained clinical pathologist, who should be afforded an opportunity personally to serve local physicians and who, for economic reasons, may supervise several neighboring laboratories within a radius of 25 or 50 miles. This should give adequate, individualized laboratory service to all sections of the state. Moreover, he should be encouraged to avail himself of the facilities of the Department of Pathology of our Medical School for consultation, and to act as advisor and supervisor of an educational program for the local physicians whom he serves.

If this proposal were carried out, it should become unnecessary for the state laboratory to perform such diagnostic procedures as the Rh determination, tumor diagnosis, and the like, which certain public health officials and socially minded physicians are advocating. It might serve as a definite deterrent to a threat of socialized medicine. It should stimulate initiative and incentive in the clinical pathologist to equip his laboratory fully in order to render such diagnostic services as the state laboratory might otherwise render.

The clinical pathologist recognizes the leadership and the ability of the Minnesota State Board of Health Laboratory in research and diagnosis, by reason of the high qualifications of its scientific personnel and the economic advantages it enjoys. He looks upon it as a consultant and final authority in public health laboratory diagnosis. He recognizes the peculiarly desirable place it commands in promoting research and publicity relating to diseases which are of utmost public concern. He appreciates the healthy influence it can exert upon the practice of medicine in general and clinical pathology in particular. Such undertakings as the serologic evaluation program for participating laboratories, currently being carried out, the statistical cancer survey service now being inaugurated, and other practical investigations dealing primarily with public health, are welcome contributions which the state laboratory can make without actually entering into the practice of medicine.

The clinical pathologist feels that where public health is not of immediate concern, clinical laboratory services should be left in his hands, and that he should not be placed in the role of a competitor in the practice of his specialty against the state operated laboratory. To burden the state laboratory with diagnostic tasks which primarily belong to the clinical pathologist might seem wise and expedient to some physicians, and a welcome sign to the advocates of socialized medicine. It would be accomplished, however, only at the sacrifice of a principle on which American medicine rests, and from which there should be no retreat.

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KANO IKEDA, M.D.

TECHNICAL SECTION

CARDIOLIPIN-LECITHIN-CHOLESTEROL ANTIGEN IN THE PRECIPITATION TEST FOR SYPHILIS

INFLUENCE OF RATIO OF LECITHIN TO CARDIOLIPIN ON ANTIGEN ACTIVITY*

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In studies on the adjustment of cardiolipin-lecithin-cholesterol as antigen in the macroprecipitation test for syphilis,² it was noted that maximum reactivity occurred when the ratio of lecithin to cardiolipin was between 10:1 and 25:1. When antigens of varying proportions within this range were tested with dilutions of a standard pool of reacting serums, the maximum reactivity seemed to depend on the total concentration of lipid rather than on the proportion of lecithin to cardiolipin. With undiluted serums, however, as the proportion of lecithin to cardiolipin was increased from 10:1 to 25:1, the tendency of high-titered serums to give prozone reactions and the inhibition phenomenon decreased.¹ Extensive use of the antigen prepared in the 25:1 ratio demonstrated the quantitative relation between serum and antigen; and the result of the test could, therefore, be expressed as a numerical titer.⁴

The present report gives further data on the differences in reactivity of two antigen mixtures:

	LECITHIN: CARDIOLIPIN	LECITHIN	CARDIOLIPIN	CHOLESTEROL
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Routine antigen.....	25:1	0.75	0.03	0.25
Experimental antigen.....	10:1	0.75	0.075	0.25

Over a period of nine months parallel tests were made with serums of different reactivities according to the routine technic.^{4, 6} High-titered serums were diluted in 0.85 per cent sodium chloride solution. Frequently, quantitative relations were not demonstrated with the experimental antigen; and, usually, the titers with it were lower than with the routine antigen—that is, the reactions with diluted and undiluted serums could not be correlated (Table 1). The reactivity of the serum was not maintained on dilution. However, when nonreacting human serum was substituted for saline as the diluent,⁵ quantitative relations were demonstrable with the experimental antigen. Occasionally, the titers of highly reacting serums were slightly lower than with the routine antigen.

Table 1 shows the reactions of 3 typical serums taken from a large number of comparative tests. Undiluted, the serums had titers greater than 5 with both

* Received for publication, February 6, 1948.

antigens. In subsequent tests of 2 of these specimens diluted in physiologic saline, the titers were confirmed only with the routine antigen. But in parallel tests of the 3 serums diluted in nonreacting serum, the titers with the experimental antigen were the same or only slightly lower than those with the routine antigen.

The failure of the experimental antigen to give quantitative results may be related to its tendency to give unstable precipitates with serum. Precipitates with the routine antigen are extremely stable and withstand hard shaking, a second reading several hours after the completion of the test agreeing with the original reading. With the experimental antigen the precipitates are less stable and sometimes even disappear on shaking. The phenomenon is relatively common in specimens that react with the experimental and not with the routine antigen and is more marked when the antigen is present in excess. When first observed,

TABLE 1

SIGNIFICANCE OF RATIO OF LECITHIN TO CARDIOLIPIN IN THE ANTIGEN FOR QUANTITATIVE PRECIPITATION TESTS

SPECIMEN NUMBER	TITERS					
	Undiluted serum		Diluted Serum			
	Routine antigen (25:1)*	Experimental antigen (10:1)*	Saline diluent†		Serum diluent‡	
			Routine antigen (25:1)	Experimental antigen (10:1)	Routine antigen (25:1)	Experimental antigen (10:1)
64467	>5	>5	15	<5	15	10
102941	>5	>5	10	<2.5	10	10
104924	>5	>5	40	30	40	40

* Ratio of lecithin to cardiolipin

† 0.85 per cent sodium chloride solution

‡ Pooled nonreacting human serums

a 3 tube test may read 4+, 4+, 4+, but after very slight agitation may become 4+, 2+, -. In such a case the principle that is applied routinely to the determination of titers does not hold. The excess of cardiolipin seems to have a dispersing effect on the precipitate in the test; moreover, tests of nonreacting serums appear much less granular than the routine tests. This may be a natural consequence of the marked peptizing action which cardiolipin in small amounts exerts on suspensions of cholesterol crystals.³

Occasionally, the experimental antigen reacts slightly with serum from a person infected with syphilis earlier in the course of the disease than does the routine antigen. The significance of these reactions with an antigen that does not give accurate quantitative information appears questionable.

SUMMARY

An experimental antigen containing lecithin and cardiolipin in a ratio of 10:1 has been compared over a period of nine months in serologic tests for syphilis with the routine antigen in a 25:1 ratio.

The experimental antigen gives more serum prozone and inhibited reactions than the routine antigen. It does not always show quantitative relations with serums that are diluted with physiologic saline. Quantitative relations are approximated, however, when nonreacting human serum is used as the diluent.

The precipitates formed in tests with undiluted serum and experimental antigen occasionally disappear on shaking, especially in the presence of excess of antigen.

The experimental antigen occasionally reacts to a slight degree with serum from a case of syphilis at an earlier stage than does the routine antigen.

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A RAPID CEPHALIN CHOLESTEROL FLOCCULATION TEST USING CENTRIFUGATION*

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In the course of a study of the various factors influencing the flocculation of cephalin and cholesterol mixtures in saline, it was found that a rapid precipitation of the flocculent material occurred with certain serums when the tubes were centrifuged. Although the centrifuge method proved to be less sensitive than the standard cephalin cholesterol flocculation test, it seemed to be of some practical value since the reading could be made at once.

METHODS

The antigen was prepared in the usual way from a stock material (Difco). Only non-hemolyzed serum obtained on the day of testing was used. The regular cephalin cholesterol flocculation test² was carried out, and at the same time 0.1 cc. of serum, 0.2 cc. of normal saline and 0.2 cc. of freshly prepared antigen were added to Kahn tubes. The amounts were arrived at after considerable experimentation with varying concentrations of saline, antigen and serum. The contents of the tubes were well mixed, and the tubes were centrifuged for three minutes at approximately 1500 r.p.m. The tests were read immediately, and the readings were graded from 0 to 4 plus.

RESULTS

In this study the serums of 269 persons were tested by the rapid centrifuge technic and the results compared with the regular cephalin cholesterol flocculation test. The serums tested came from patients with acute and chronic liver diseases, extra-hepatic biliary tract obstruction, miscellaneous diseases and from normal controls.

A. Cirrhosis of the Liver

In a group of 42 patients with cirrhosis of the liver, 32 had cirrhosis of the Laennec type. In all of these patients the Hanger test was strongly positive and the rapid test was less sensitive (Table 1). This group of cirrhotics had the disease in an active phase. The results of the 2 tests were in agreement in all 5 patients with biliary cirrhosis and in 6 of the 7 patients with unclassified cirrhosis.

B. Hepatitis

In another group of 38 patients, there were 27 with acute infectious hepatitis, 5 with convalescent hepatitis and 11 with infectious mononucleosis. As noted in Table 2, the standard test gave a higher percentage of positive reactions as

* Received for publication, December 1, 1947.

compared with the rapid test in acute infectious hepatitis; but, in convalescent hepatitis both tests tended to be negative. In infectious mononucleosis, where

TABLE 1

COMPARISON OF RESULTS OF CEPHALIN CHOLESTEROL FLOCCULATION TESTS BY HANGER'S METHOD AND BY CENTRIFUGE METHOD IN 42 PATIENTS WITH CIRRHOSIS OF THE LIVER

TYPE OF CIRRHOSIS	NO. OF PATIENTS	CENTRIFUGE METHOD					REGULAR METHOD				
		0	1+	2+	3+	4+	0	1+	2+	3+	4+
Laennec's.....	30	3	1	4	10	12	0	0	0	12	18
Biliary.....	5	2	0	0	1	2	2	0	0	1	2
Unclassified.....	7	3	1	0	0	3	2	1	0	0	4
Total.....	42	8	2	4	11	17	4	1	0	13	24

TABLE 2

COMPARISON OF RESULTS OF CEPHALIN FLOCCULATION TESTS BY HANGER'S METHOD AND CENTRIFUGE METHOD IN 27 PATIENTS WITH HEPATITIS AND IN 11 PATIENTS WITH INFECTIOUS MONONUCLEOSIS

TYPE	NO. OF PATIENTS	CENTRIFUGE METHOD					REGULAR METHOD				
		0	1	2	3	4	0	1	2	3	4
Acute hepatitis.....	22	4	0	2	7	9	1	0	1	7	13
Convalescent hepatitis.....	5	3	2	0	0	0	2	2	0	1	0
Infectious mononucleosis.....	11	4	0	1	3	3	3	0	0	4	4
Total.....	38	11	2	3	10	12	6	2	1	12	17

TABLE 3

COMPARISON OF RESULTS OF CEPHALIN FLOCCULATION TESTS BY HANGER'S METHOD AND CENTRIFUGE METHOD IN 10 PATIENTS WITH OBSTRUCTIVE JAUNDICE

TYPE	NO. OF PATIENTS	CENTRIFUGE METHOD					REGULAR METHOD				
		0	1+	2+	3+	4+	0	1+	2+	3+	4+
Stone.....	4	4	0	0	0	0	4	0	0	0	0
Stricture.....	1	1	0	0	0	0	1	0	0	0	0
Carcinoma of pancreas.....	3	2	0	1	0	0	3	0	0	0	0
Cholangitis.....	2	2	0	0	0	0	1	0	1	0	0
Total.....	10	9	0	1	0	0	9	0	1	0	0

hepatitis is known to be a common finding,¹ both tests gave positive results in the majority of cases.

C. Obstructive Jaundice

If obstruction of the bile ducts persists long enough, liver cell damage will occur and flocculation tests often become positive. In this study (Table 3) 10

patients whose jaundice was due to extra-hepatic obstruction as proved at operation or autopsy, gave uniformly negative reactions in both tests.

D. Miscellaneous Diseases

Fifty-eight patients with miscellaneous diseases were tested. In patients with disseminated lupus erythematosus, strongly positive reactions were obtained with both tests, and this was true also of one patient with malaria. In both of these diseases the cephalin cholesterol flocculation test is usually strongly positive.⁴ One patient with carcinoma of the sigmoid without liver metastasis also gave a positive reaction to both tests. In the remaining patients the positive tests occurred in conditions not usually associated with diffuse liver disease and not commonly known to have positive flocculation tests. In this regard the standard test gave a higher number of "false positive" reactions than the rapid test (Table 4).

TABLE 4

COMPARISON OF RESULTS OF CEPHALIN FLOCCULATION TEST BY HANGER'S METHOD AND THE CENTRIFUGE METHOD IN 58 PATIENTS WITH MISCELLANEOUS DISEASES AND IN 121 NORMAL CONTROLS

TYPE	NO. OF PATIENTS	CENTRIFUGE METHOD					REGULAR METHOD				
		0	1+	2+	3+	4+	0	1+	2+	3+	4+
Miscellaneous.....	58	49	2	1	2	4	45	0	7	3	3
Controls.....	121	118	1	1	1	0	110	0	5	4	2

E. Normal Controls

There were 121 healthy persons who served as controls. This group was composed mainly of medical students and internes who gave no history or clinical evidence of liver disease. In one person both tests were strongly positive (3 plus reaction), and the laboratory data indicated the presence of hepatitis. No other person gave a strongly positive reaction with the centrifuge method, but 5 others gave positive reactions with the standard test (Table 4).

COMMENT

It is well known that the cephalin cholesterol flocculation test of Hanger is not specific for liver disease. While the cause of the flocculation is undoubtedly bound up with an alteration of serum proteins, recent investigation has failed to demonstrate the exact nature of this phenomenon.^{3, 4} The question also has been raised as to whether the altered serum protein is due to a product of a damaged liver or whether it is of the nature of an immune antibody. It is of interest that centrifugation accelerates flocculation with certain abnormal serums much in the fashion that antibody-antigen reactions are enhanced by the same procedure.

Although the standard cephalin cholesterol flocculation test has a number of disadvantages, it is a useful laboratory procedure. In spite of a meticulous

technic, flocculations occasionally occur with serums from individuals free of laboratory and clinical evidence of liver disease or other disorders known to produce positive tests. A study was made of the influence on flocculation of various factors, including changes in pH, salt concentration, temperature and centrifugation. The centrifuge test is less sensitive than the regular test but in this study produced fewer "false positives". As in the regular Hanger's test, fresh serum and freshly prepared antigen and normal saline solution were essential for accurate results. However, the fact that the test can be read within a few minutes, rather than after twenty-four or forty-eight hours, seems to warrant a trial of its use. At the same time it is recommended that the regular cephalin cholesterol flocculation test be carried out until further experience indicates whether this rapid test is dependable.

SUMMARY

In the course of studying various factors influencing the flocculation of cephalin cholesterol mixtures, it was discovered that centrifuging often enhanced the reaction. While less sensitive than Hanger's test, the centrifuge technic is rapid, permitting the reading of the test within a few minutes. Further investigation as to the usefulness of this procedure seems indicated.

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A COMPARISON OF SIMMONS' SLIDE METHOD AND CHOWN'S CAPILLARY TUBE METHOD FOR THE DETECTION OF THE Rh FACTOR*

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The routine testing of the blood of large numbers of patients for the Rh factor has necessitated the use of procedures which will give reliable results quickly. Wiener's tube method,⁶ Diamond and Abelson's slide method³ and Simmons' slide method⁵ have all been used in this laboratory.

In Wiener's method the cell pattern is used to determine whether the patient's red blood cells have been agglutinated by the anti-Rh testing serum; the sediment is examined microscopically in cases in which agglutination cannot be clearly seen by reading the sediment pattern. This procedure requires an experienced worker.

We have tried Diamond and Abelson's method using the thick cell suspension (50 per cent in serum) and have also found it difficult to read because of drying at the edges of the cell-serum mixture and the tendency for rouleaux formation.

Simmons' method which employs a 5 per cent cell suspension, has been used on thousands of blood specimens in conjunction with the Wiener tube method and has proved to be very reliable. It is now the procedure of choice for routine examination of red cells for the Rh factor in this hospital. In the present study we have compared Chown's capillary method² with Simmons' slide method in testing 150 different red blood cell suspensions.

DESCRIPTION OF TECHNIQS

Simmons' Slide Method

Glass slides, 4 inches by 3 inches, divided into 12 rectangles by thick painted lines, are used in this test. One drop of potent anti-Rh₀ serum, adsorbed to remove anti-A and anti-B factors, is placed in one rectangle together with one drop of a 5 per cent suspension of the patient's red blood cells suspended in Rous-Turner solution.† Suspensions of known Rh-positive and Rh-negative red blood cells are mixed with the same anti-Rh₀ serum in each of two other rectangles. After mixing, the slide is placed in a moisture chamber and incubated at 37 C. for thirty minutes. It is advisable to avoid disturbance of the slide until readings are to be made. If the slide is then gently rocked so as to rotate the mixture in a clockwise direction, the smooth cell serum areas tend to break up in large aggregates when the Rh agglutinin is present in the cells. When no Rh substance is present, the cell serum area shows no sign of agglutination.

* Received for publication, October 31, 1947.

† Rous-Turner mixture consists of (1) 16 cc. of 5.4 per cent glucose in distilled water and (2) 6.6 cc. of 3.8 per cent sodium citrate in distilled water. Each solution is sterilized at 110 C. for fifteen minutes, and then the two solutions are mixed together.

If the slide is shaken roughly, the agglutination in positive tests tends to break up into very small aggregates sometimes difficult to detect. A watchmaker's lens can then be used to verify the absence of agglutination. If the observer is in doubt as to whether fine granulation represents slight agglutination or rouleaux formation, microscopic examination is used for a final decision.

Apart from emergency work, when one cell suspension and appropriate controls are examined, the method has three advantages for routine testing of large numbers of specimens: (1) Ten samples can be tested on one slide; (2) a number of such slides can be set up and incubated in rapid rotation and the results read in similar rotation. When a number of tests are being set up at one time, the controls can be included on the first slide and the later slides used in comparison with them; (3) the time taken to prepare 12 cell suspensions, set up the Rh test and read the results after the incubation period does not exceed nine minutes, in addition to the thirty minute incubation period.

Chown's Capillary Tube Method

In this method the end of a capillary tube 0.5 mm. by 10–15 cm. is placed in anti-Rh testing serum and filled to the length of 2 cm. The tube is then placed in a 10 to 20 per cent serum suspension of patient's red blood cells. After mixing the constituents and sealing the end of the tube, it is placed in a rack arranged so that the tubes slope at an angle of 45 degrees to the base. After incubation for thirty minutes at 37 C., the tubes are read against a white background in bright light. Coarse agglutination along the tube indicates the presence of the Rh factor, and a smooth line of sedimented cells shows that it is absent.

METHOD AND RESULTS

Independent readings of these two tests were made by two workers on 150 different suspensions of red blood cells. The capillary tube method was read first to avoid bias in favor of the test with which we were most familiar.

The results of the two tests on these 150 different blood samples agreed in 92 cases, but showed discrepancy in 58 samples. In 34 of the latter the reading of the Chown test was the same by both observers, but it did not agree with the result of the Simmons' method. Twenty-nine samples in this group were Rh-positive according to Chown's test but Rh-negative with Simmons' test. The other 5 samples were Rh-negative by Chown's test but Rh-positive by Simmons' test. There was a difference of opinion between the two observers in the reading of Chown's test on 24 specimens. In 17 of these cases Simmons' test was positive. One of us (K.) read Chown's test as positive in 6 cases, as negative in 2 cases and was undecided about the reading in 9 cases. The corresponding figures for the other observer (W.) were 4, 11 and 2. The 7 instances that were negative by Simmons' test were read in Chown's test by K. as negative in 3, positive in 2 and undecided in 2, while the corresponding readings were made by W. in 2, 3 and 2 cases. The distribution of these figures shows that there was no deviation in any definite direction, so it is unlikely that personal factors were involved.

DISCUSSION

There is no doubt in our minds that when a discrepancy occurred between the Chown and Simmons' tests, the reading of the latter test was the correct one. We have tested this method on over 17,000 different specimens of blood against Wiener's tube method, in which the result is read by the sediment pattern, and have found consistent agreement.⁴ In both of these methods microscopic examination of the sedimented cells had to be made in a few instances in which there was some doubt as to the presence or absence of agglutination.

Chown's capillary tube method for the detection of the Rh factor proved to be unsatisfactory in our hands, because so many tests were read as positive when they were really negative. The chief difficulty seemed to be that very few specimens settled into a "smooth line of sedimented cells" as described by Berlin.¹ On the other hand, such an appearance did sometimes occur in tests on blood containing the Rh factor. After some handling, the whole length of cells shrank away from the tube walls and was seen to consist of solid clot. This led to some positive specimens being reported as Rh-negative.

From our observations Chown's test cannot be regarded as a safe method for detection of the Rh factor in the hands of inexperienced workers, while Simmons' slide technic has already proved very satisfactory for routine work, even by young technicians. We are satisfied that blood specimens which contain no Rh substance will not be reported as Rh-positive and that only a few specimens in which the Rh factor reacts very weakly with even potent anti-Rh₀ serum are likely to be classified as Rh-negative. These errors are likely to be detected, since it is our custom to test all cells against two potent anti-Rh₀ serums and to subtype all cells from blood which is to be used for blood transfusions or from patients whose histories give any indication that they may have previously been immunized to the Rh factor.

SUGGESTED METHOD FOR RAPID TESTING IN EMERGENCY TRANSFUSIONS

Using one of the Simmons' slides, mix the patient's red cell suspension with typing serums group A, group B and group O, respectively, in the three rectangles across the narrow side of the slide. The blood grouping is checked by mixing the patient's serum with group A and group B cells in the second row of rectangles. Rh typing of the patient's cell suspension against two adsorbed, potent anti-Rh serums can be done in the third row of rectangles, while the patient's serum can be cross-typed with two sets of group O Rh-positive and one set of group O Rh-negative cells in the bottom row. A second slide is used for controls which include an Rh-positive and an Rh-negative cell suspension each tested against the two potent, adsorbed anti-Rh testing serums.

The slides are placed in a moisture chamber and can be inspected after incubation at 37 C. for fifteen minutes. With some specimens of blood, grouping, Rh typing and cross matching can be decided in this short space of time. Inspection after incubation for a further period of fifteen minutes gives a final decision regarding the patient's blood type and suitable donor.

SUMMARY AND CONCLUSIONS

Simmons' slide method and Chown's capillary tube method for the detection of the Rh factor were compared in tests on 150 red blood cell suspensions.

A rapid method for simultaneous determination of the blood group and Rh type, and selection of a suitable group O donor for emergency transfusions is suggested.

In our series of tests Chown's capillary method has not proved satisfactory, even in the hands of experienced workers. Simmons' slide method is much simpler and far more reliable and, provided a reasonably potent anti-Rh testing serum is available, can be used by relatively inexperienced persons.

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DETERMINATION OF SERUM CALCIUM BY TURBIDIMETRY*

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The method of Clark and Collip,¹ which involves a potassium permanganate titration of precipitated calcium oxalate, generally is used to determine serum calcium concentrations. While results obtained by this method are reproducible, it is tedious and time consuming and requires considerable technical skill. A simpler and more rapid technic was sought, therefore, which would be accurate enough for clinical purposes. Such a method, employing turbidimetry, is presented.

Method. When serum calcium is precipitated by ammonium oxalate, an evenly turbid suspension of fine homogeneous calcium oxalate crystals is formed. The light absorption of this suspension can be measured easily and quickly with any of the commercial photoelectric colorimeters. Since serum calcium values vary within narrow limits, even under pathologic conditions, the light absorption of the turbid suspension conforms to Beer's law in the same manner as that of a colored solution.

One cc. of serum is added to each of two colorimeter tubes. The serum in the first tube is diluted to 10.0 cc. with distilled water. This tube is used as a blank to compensate for any intrinsic light-absorbing qualities of the serum itself and corrects for hemolysis, icterus or lipemia. To the serum in the second tube is added 2.0 cc. of saturated ammonium oxalate solution and 7.0 cc. of distilled water. The tubes are set aside for at least ten minutes to assure complete precipitation of the calcium. The blank tube is placed in the colorimeter, and the scale is set to read "100". Then a reading is obtained from the tube containing the turbid suspension of calcium oxalate. The serum calcium concentration in mg. per 100 ml. of serum then is determined from a standard reference curve.

In this laboratory a standard reference curve was constructed by the following procedure:†

Dilutions of a standard solution of calcium chloride in distilled water were prepared so that resulting samples contained respectively 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 mg. of calcium per 100 ml. Serum, from which calcium had been removed by centrifugation after the addition of solid ammonium oxalate, was used

* Received for publication, February 24, 1948.

† A simpler method can be used to prepare a similar curve. Approximately 25 mg. of calcium chloride is added to 50 cc. of pooled serum, thereby assuring a final concentration in excess of 20 mg. per 100 ml. The total concentration is determined by the method of Clark and Collip. Nine dilutions of the serum are made so that the resulting samples contain respectively 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 mg. of calcium per 100 ml. These samples are substituted for unknown serum, and the procedure for determining calcium concentration turbidimetrically is carried out as described above.

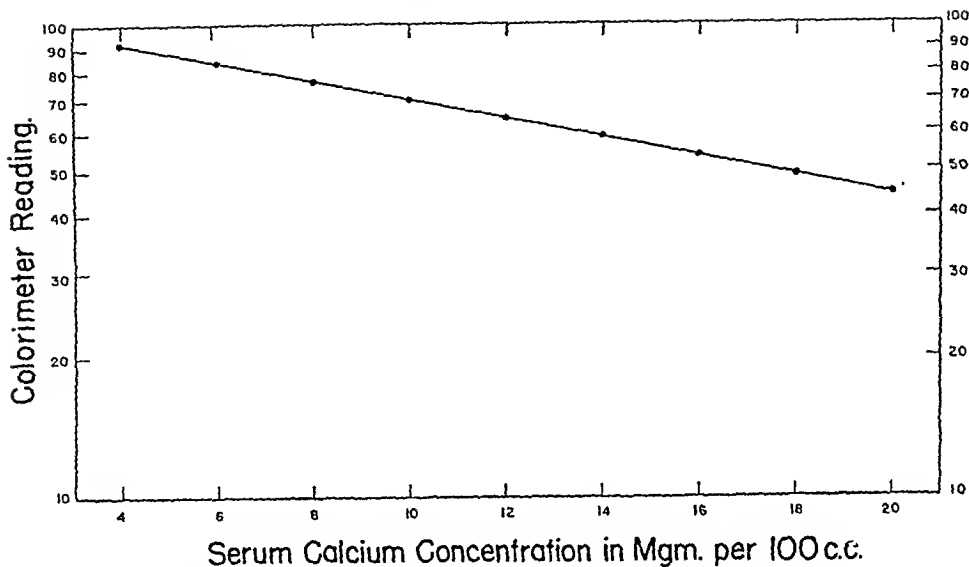


FIG. 1. Standard reference curve, known values for serum calcium concentration are plotted against colorimeter readings.

TABLE 1

COMPARISON OF SERUM CALCIUM CONCENTRATION BY TURBIDIMETRIC METHOD AND METHOD OF CLARK AND COLLIP IN 20 PERSONS

SAMPLE OF BLOOD	SERUM CALCIUM CONCENTRATION IN MG. PER 100 ML.		PER CENT DIFFERENCE
	Method of Clark and Collip	Turbidimetric Method	
1	11.0	11.3	+3
2	8.4	8.3	-1
3	9.0	9.0	0
4	10.7	10.4	-3
5	9.4	9.5	+1
6	8.7	8.9	+2
7	10.1	10.2	+1
8	6.8	7.0	+3
9	8.5	8.5	0
10	8.6	8.4	-2
11	8.0	7.9	-1
12	10.2	10.1	-1
13	9.0	9.3	+3
14	8.5	8.2	-4
15	7.6	7.8	+3
16	9.2	9.0	-2
17	10.1	10.4	+3
18	8.0	8.3	+4
19	9.2	8.9	-3
20	9.3	9.0	-3
			Mean difference = 0

for the determination. In each instance 1.0 cc. of one of the standard calcium dilutions was substituted for 1.0 cc. of distilled water used in the described

procedure. Readings obtained from the resulting turbid suspensions were plotted on semilogarithmic graph paper, as shown in Figure 1. Since characteristics of various makes of photoelectric colorimeters differ, it is necessary to prepare a separate curve for each instrument.*

Results. As is shown by Figure 1, an accurate linear relationship was found to exist between the known calcium content of the serums and the colorimeter readings. In order to test further the accuracy of the turbidimetric method, the serum calcium concentration of each of 20 different individuals was determined both by the turbidimetric method and by the titration procedure of Clark and Collip. The results, presented in Table 1, reveal a close relationship between the two methods.

Comment. The turbidimetric method described here for determining serum calcium concentration is consistently accurate, and the results compare favorably with values obtained from the same specimens by the method of Clark and Collip. Although reasonable care should be exercised in performing the determinations, no especial technical skill is necessary; and the entire procedure may be accomplished within a few minutes.

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* The instrument used to obtain the curve shown in Figure 1 was a Coleman Model II Spectrophotometer.

IMPROVED CONCENTRATION METHOD FOR BACTERIA, INCLUDING TUBERCLE BACILLI*

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It is often desired to concentrate bacteria present in urine or in fluids from serous cavities before smears and cultures are made. This is especially important when the bacteria are scarce.

Centrifugation alone is often unsatisfactory, since many bacteria fail to sediment unless a large and powerful centrifuge is employed. In the method here proposed, one adds to the fluid to be examined about one-tenth of its volume of 2 per cent neutral sodium oxalate. (Twenty Gm. C. P. sodium oxalate is dissolved in 1000 cc. of distilled water, distributed in test tubes and autoclaved. Each tube is used once or twice only.) The oxalate combines with the calcium that is always present in biologic fluids and slowly forms a very fine and homogeneous fog of calcium oxalate which precipitates gradually, carrying the bacteria down with it. The tubes are then centrifuged, the supernatant fluid discarded and the sediment used for culture and smears. This method has proved successful for the isolation of bacteria from urine, cerebrospinal fluid and fluids from serous cavities.

METHOD

Mix the test material with three times its volume of digesting solution (acid or alkali) in a test tube. In order to avoid wetting the stopper during shaking, the tube is best stoppered with nonabsorbent cotton wool. Shake well and incubate at 37 C. for from thirty to forty-five minutes. Then centrifuge, discard the supernatant, shake the sediment and neutralize with the alternate (acid or alkaline) solution, added drop by drop.

When the alkaline digest is being neutralized with the acid solution, a tertiary alkaline sodium phosphate (Na_3PO_4) is formed by the interaction of the phosphoric acid and excess sodium hydroxide. On further addition of acid, this compound is gradually converted into the secondary less alkaline salt (Na_2HPO_4), which in turn is partly transformed into the primary acid phosphate (NaH_2PO_4). The mixture of acid and alkaline phosphates possesses strong buffering properties so that an excess of a few drops of acid near the neutral point does not change the pH materially.

When the acid digest is being neutralized with the alkaline solution, the same thing happens except that the phosphates are formed in reverse order, starting with the appearance of the primary salt and followed by partial transformation into the secondary compound.

The proposed indicator has the advantage of three distinct color changes corresponding to the alkaline, neutral (pH 6.0-8.0) and acid zones, the respective

* Received for publication, November 1, 1947.

colors being blue, yellow and red. Thus, it can be conveniently employed either in acid or alkaline digestion, the neutralization being carried on until the blue or red is transformed into yellow.

The neutralized material is transferred to the medium preferred by the laboratory. If no visible growth appears within two or three weeks, the medium is scraped with the platinum loop and smears are prepared and examined in the usual way. The culture is incubated for an additional two weeks, when a visible growth may be expected, owing to the possible spreading of microscopic colonies on the medium by scraping with the loop.

In specimens suspected of harboring *Salmonella* or typhoid bacilli, an enriching fluid (sodium tetrathionate or selenite sodium) can be added to the sediment.

CONCENTRATION METHOD FOR TUBERCLE BACILLI

In looking for tubercle bacilli the method is modified, depending on whether the material is secondarily contaminated or not. Noncontaminated material, such as cerebrospinal fluid, pleural fluid or catheterized urine, is treated directly with sodium oxalate and the sediment cultured. However, in case of secondarily contaminated material, the sediment, on addition of sodium oxalate, is first treated with acid or alkali in fairly strong concentration in order to kill the less resistant micro-organisms. We have found it best to employ acid or alkali on separate samples since occasional strains of *Mycobacterium tuberculosis* may be unusually sensitive either to acid or alkali.

As the alkaline or acid reaction inhibits the development of tubercle bacilli in culture, the treated material must be brought near neutrality before inoculation. This is the most difficult part of the technic and can be accomplished either by means of repeated washing or by direct neutralization. The first method has the disadvantage of frequent contaminations, while direct neutralization is difficult because of the relatively concentrated solutions employed, the neutralizing solution being frequently added in excess, even when pH indicators are added.

We succeeded in overcoming the difficulties of the direct neutralization method by combining the use of a suitable buffer and a convenient indicator for the proper pH (6.0 to 8.0). The reagents to be employed are:

1. Methyl-red thymol-blue indicator¹ which is made up of 10 mg. methyl-red and 70 mg. thymol-blue, both dissolved in 100 cc. N/10 NaOH.
2. Digesting and neutralizing solutions:

Acid solution. Add concentrated sulfuric acid, 2 cc., and concentrated phosphoric acid (H_3PO_4) 2 cc. to 50 cc. water. Cool, add 10 cc. of the indicator and fill to 100 cc.

Alkaline solution. A 4 per cent aqueous solution of sodium hydroxide containing 10 cc. of the indicator for every 100 cc. of material.

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MICROESTIMATION OF THE WELTMANN COAGULATION REACTION*

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We wish to describe the estimation of the Weltmann coagulation reaction in small quantities of serum and also mention some improvements in the preparation of the solutions. The Weltmann coagulation reaction may be used as an aid in the differential diagnosis of certain diseases.¹ The principle of the test is based on the property of serum proteins to coagulate in the presence of different amounts of calcium chloride when diluted 50 times with distilled water.

A correct estimation depends on the proper preparation of the reagents. The difficulty in preparing the reagents is caused by the hygroscopic quality of the calcium chloride. If fused anhydrous calcium chloride is used, it may contain basic calcium chloride which is created by the process of fusion with loss of chloride. Consequently, the fused salt is not a reliable reagent. In order to dispense with weighing the exact amount of calcium chloride, the following method may be used.

Prepare a concentrated solution of C. P. calcium chloride ($\text{CaCl}_2 \cdot 6 \text{H}_2\text{O}$). Estimate the amount of chloride by means of the usual silver nitrate titration. The Weltmann stock solution should be diluted until 1 cc. reacts with 9.05 cc. of $\text{N}/10 \text{ AgNO}_3$.

Example. One cc. of the CaCl_2 solution corresponds to 11.2 cc. $\text{N}/10 \text{ AgNO}_3$. This solution is too concentrated and has to be diluted according to the following equation:

$$9.05:11.2 = X:1000$$
$$X = 808$$

Of the concentrated solution, 808 cc. must be diluted with distilled water to 1000 cc. which now represents the Weltmann stock solution.

In ten 100 cc. volumetric flasks are placed 1 cc., 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2 and 0.1 cc., respectively, of the stock solution; and the flasks are filled to the mark with distilled water. These flasks then contain the Weltmann solutions 1 to 10, flask 1 having the highest and flask 10 the lowest concentration of calcium chloride.

PROCEDURE

With a serologic pipet, graduated in one-hundredths cc. and bent near its tip,³ place 0.02 cc. of serum on the bottom of each of 10 small test tubes and to each add 1 cc. of the respective solutions of calcium chloride. Boil for fifteen minutes and observe the flocculation.

* Received for publication, February 9, 1948.

For serial estimations or in case there is only a small amount of serum available, it is sufficient to work with 6 test tubes. Into the first tube the mean concentration (No. 5) is placed and the reaction read after fifteen minutes' boiling. If flocculation appears, the band is continued in the right direction (6 to 10), otherwise in the left direction (4 to 1).

If the Weltmann coagulation band is determined only occasionally, it is sufficient to prepare a Weltmann solution by preparing a 1:100 dilution of the 5 per cent stock solution and making dilutions 1 to 10 in the following way:

Weltmann solution.....	1	2	3	4	5	6	7	8	9	10
5 per cent stock solution, cc.....	1.0	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1
Distilled water, cc.....	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9

Otherwise, the procedure is exactly as described above. Calcium chloride may be replaced by calcium lactate or calcium gluconate, both of which have the advantage of being obtainable as dry powder so that the weighing of an exact amount is not difficult.

1.39 Gm. of calcium lactate or 2.019 Gm. of calcium gluconate per 100 ml. correspond to a 1 to 10 dilution of the Weltmann stock solution. The Weltmann solutions 1 to 10 are prepared by measuring into each 100 cc. volumetric flask amounts from 10 cc. to 1 cc. respectively, of the stock solution and filling to the mark with distilled water.

Calcium lactate and calcium gluconate readily deteriorate as a result of the action of molds and must, therefore, be stabilized by adding strips of filter paper containing mercuric iodide which are prepared in the following manner: Powdered mercuric iodide is mixed with liquified paraffin, and this mixture is poured over filter paper. The mercuric iodide is fixed on the filter paper and serves as an excellent bacteriocide.²

SUMMARY

A microestimation of the Weltmann coagulation band is described, using 0.12 cc. to 0.2 cc. of serum.

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PREPARATION OF BARIUM AND SODIUM SALTS OF P-NITRO-PHENYLPHOSPHATE FOR SUBSTRATE FOR SERUM PHOSPHATASE DETERMINATIONS*

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Shortly after the publication of a procedure for the estimation of serum acid phosphatase using p-nitrophenylphosphate as the substrate,¹ the reagent became unavailable commercially because of production difficulties. We, therefore, attempted to prepare both the barium and the sodium salts according to the general procedure of King and Nicholson³ and tested the efficacy of the barium salt in the substrate since it was more readily prepared than the sodium salt.

Preparation of the barium p-nitrophenylphosphate. One tenth mol (13.9 Gm.) of p-nitrophenol dissolved in 50 ml. of pyridine was added slowly and with vigorous stirring to 9.0 ml. of phosphorus oxychloride. The mixture became hot and there was a vigorous evolution of fumes. After cooling until the pyridine hydrochloride crystallized, water was added to the mixture dropwise until no vigorous reaction ensued, and then more rapidly until a total of 150 ml. was added. During the subsequent addition of an equal volume of 95 per cent alcohol, a copious yellow precipitate of the barium salt of p-nitrophenylphosphate was formed. After standing overnight in the icebox the crystals were filtered off with suction, washed with 95 per cent alcohol and dried over sulfuric acid *in vacuo*. The yield of the crude barium salt was 36 Gm.

Preparation of disodium p-nitrophenylphosphate. Although King and Nicholson³ give no specific directions for the conversion of the barium salt to the sodium salt, it was assumed that their procedure for making disodium phenylphosphate from the barium phenylphosphate might be used. This proved practicable although the yield was never greater than 30 per cent of the theoretical value. The product varied in color from cream to white and had the same activity as the commercial material (Eastman Kodak Company).

Barium p-nitrophenylphosphate as a substrate for acid and alkaline phosphatase estimation. An 0.8 per cent solution of the barium salt was prepared in 0.001 N HCl. The solution which contained some undissolved material was filtered and extracted with ether in order to remove free p-nitrophenol. The buffered substrates for both the acid¹ and the alkaline² phosphatase determinations were prepared and tested with a number of serums. The results agreed well with those obtained when the same serums and substrates prepared from commercial disodium p-nitrophenylphosphate (Eastman Kodak Company) were used.

SUMMARY

Barium as well as sodium salt of p-nitrophenylphosphate may be used as the substrate for the determination of acid and alkaline phosphatase in serum. The general procedure for the preparation of the barium and sodium p-nitrophenylphosphates is given.

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A SIMPLY-CONSTRUCTED MICRO-EXTRACTOR FOR BLOOD ANALYSIS*

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The apparatus to be described utilizes a modified cold-finger condenser which permits the rapid and continuous extraction of blood constituents by recondensing the extracting fluid directly above the blood specimen. A small bulb at the base of the extraction flask holds up to 20 ml. of extracting fluid. The blood specimen is placed on a small filter disc, and vapors of the boiling fluid are by-passed through four indentations in the neck of the flask which also serve to hold the filter disc in place.

Simple in design and construction, the entire apparatus can be made utilizing elementary glass-blowing technics.

CONSTRUCTION OF THE EXTRACTION JACKET

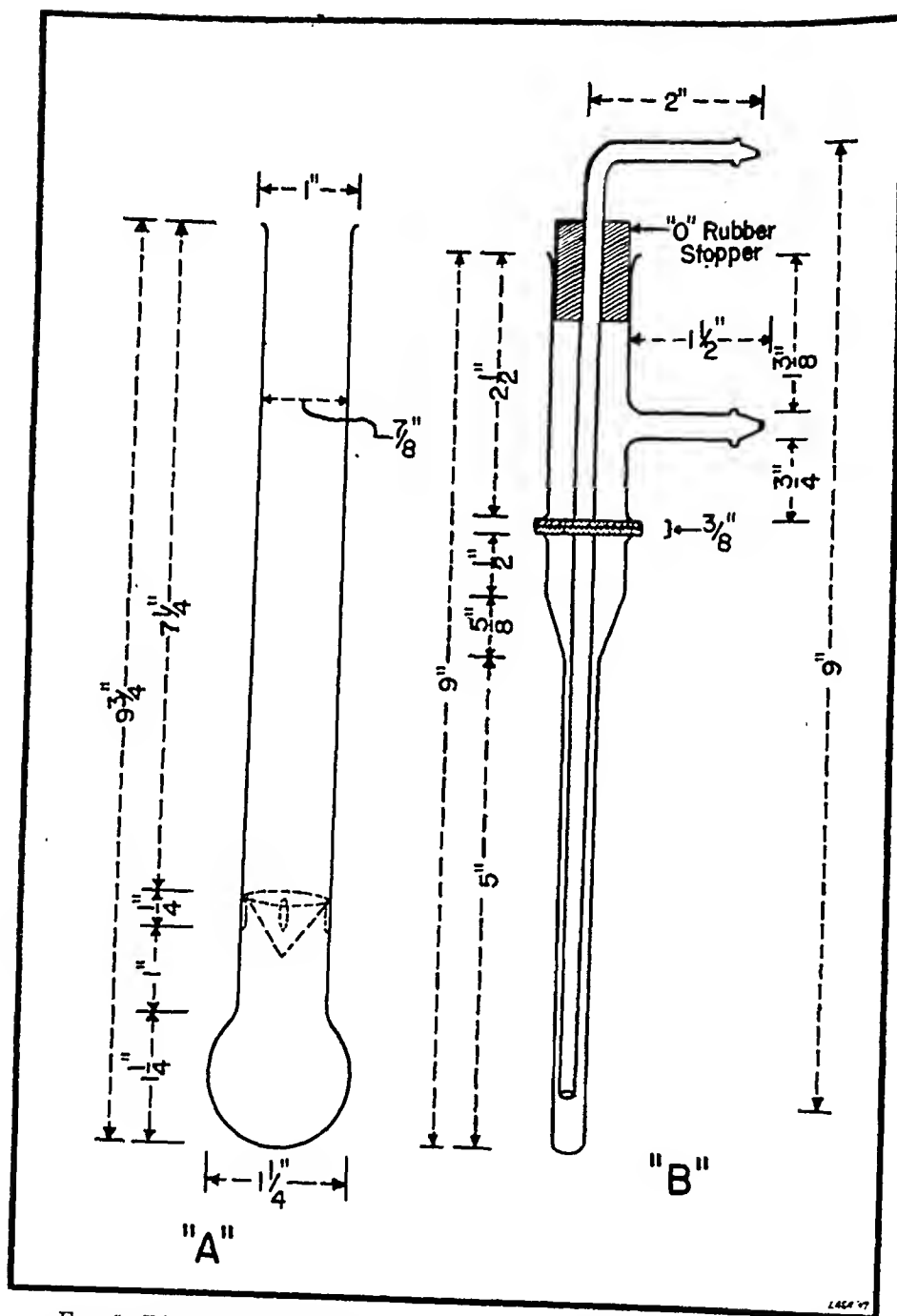
The extraction jacket (Fig. 1) consists of a single glass tube having several indentations for holding a filter disc or pad, an enlarged bulb at the base for containing the extracting fluid, and a flanged neck for cradling the modified cold-finger condenser. It is constructed of 22 mm. ($\frac{7}{8}$ ") diameter, seamless Pyrex tubing, with a wall thickness of 1 mm.

The condenser is assembled by placing the water inlet tube within the cold-finger so that the stopper holds the tip of the inner tube at a point one-half inch distant from the sealed end of the condenser. Lengths of rubber tubing are connected to the water inlet and outlet tubes, and to a water supply and drain. Heat for extractions involving an inflammable solvent may be provided by a small substage microscope lamp, with glass filter removed. This is adequate for boiling ether, acetone, ether-alcohol and acetone-alcohol mixtures. In operations which do not involve an inflammable extraction fluid, a small micro-burner or Bunsen-heated sand bath is used.

The apparatus is well adapted for micro-extractions with a small quantity of fluid, and for micro-estimations of whole blood constituents which utilize a small sample of blood. Blood specimens are placed on a small disc of heavy filter paper, or small circles cut from a filter pad and dropped into the neck of the extraction flask to rest on the small glass indentations. The boiling extraction fluid recondenses on the cold-finger, and falls directly onto the blood specimen being extracted. The condenser is sturdy in design, and provides an efficient seal by cradling, thus eliminating the use of ground-glass or rubber connections. The entire apparatus is easily dismantled for cleaning. (See Fig. 2.)

Acknowledgments. I wish to thank Robert M. Emrich for suggestions as to design of the condenser, Oliver T. Kuzma for criticism of the manuscript, and Wilfred Haflinger for photographs of the extractor.

* Received for publication, October 30, 1947.



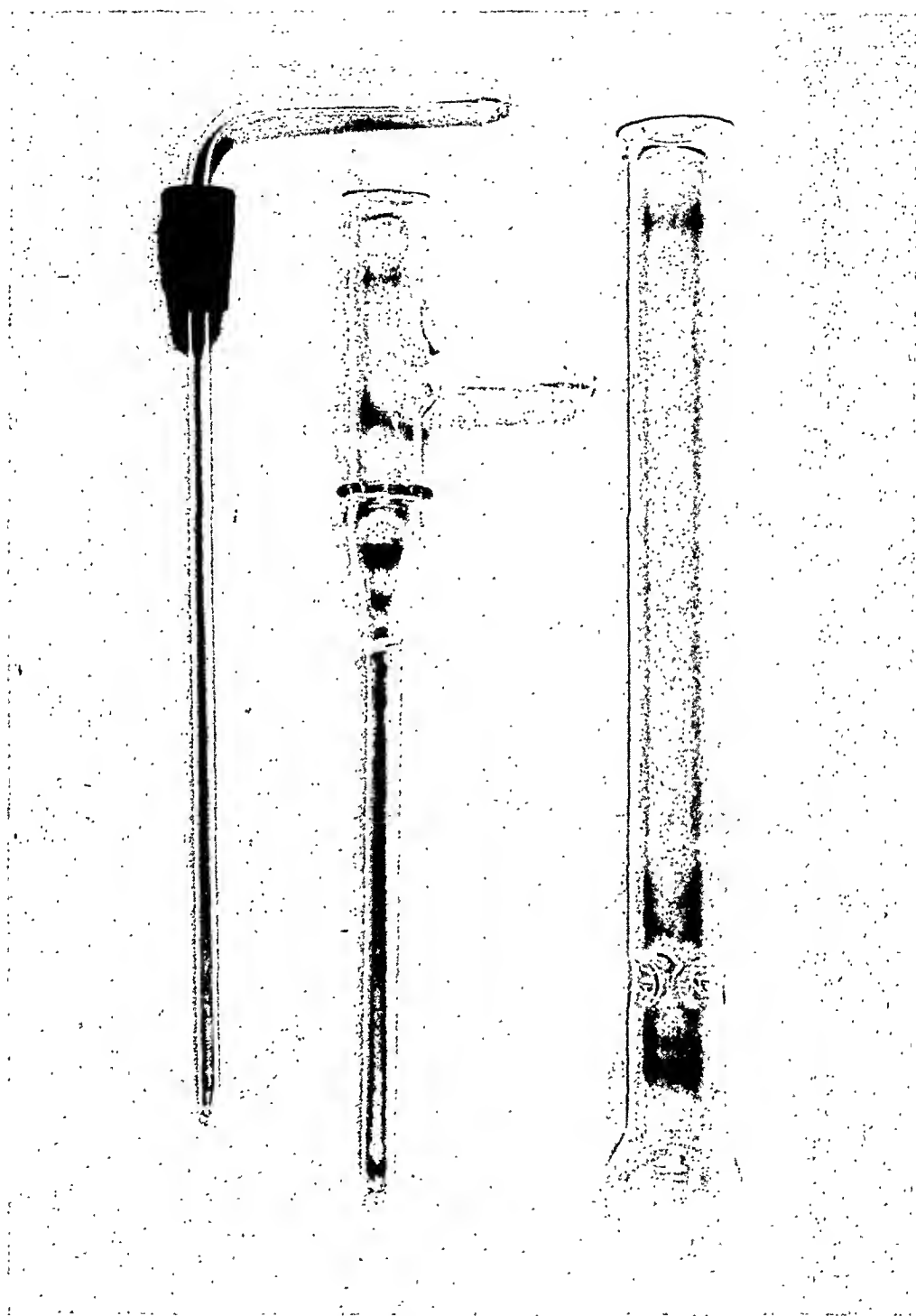


FIG. 2. Extractor parts, showing water inlet tube with rubber stopper in position, cold-finger condenser, and outer extraction tube and flask.

TEST TUBE SEALED TO HEN'S EGG FOLLOWING INOCULATION*

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Sealing of the hen's egg after inoculation with viruses, rickettsiae or bacteria, is at present accomplished by covering the opening with "Scotch" tape, cellophane

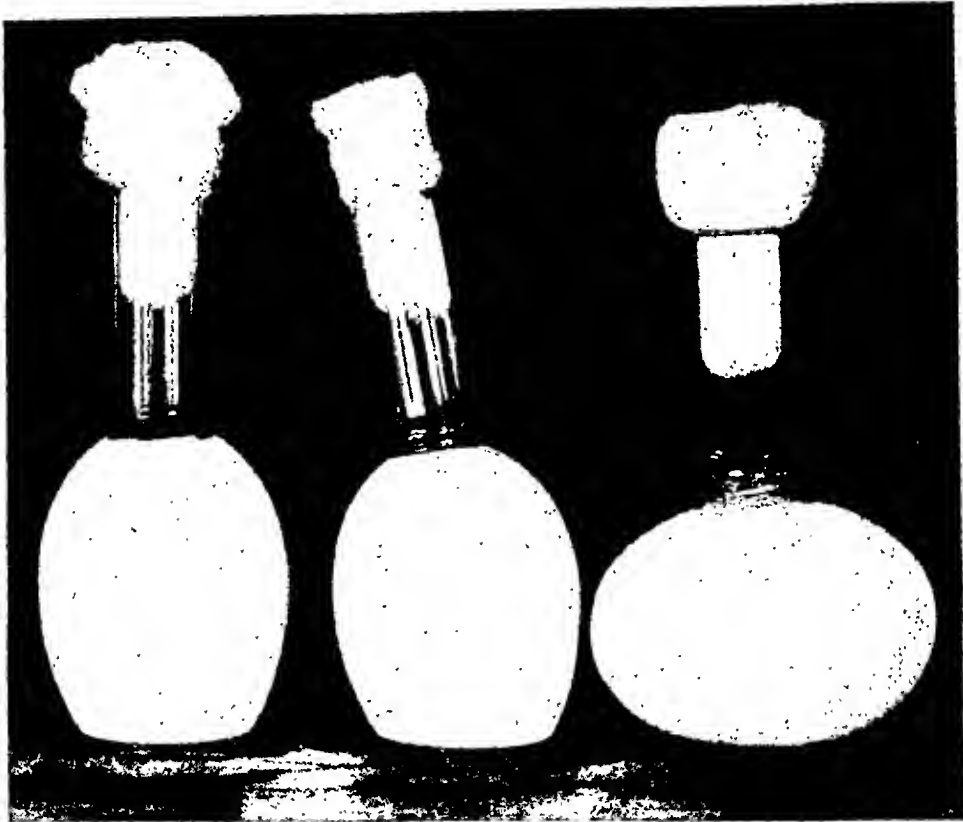


FIG. 1. Attachment of test tubes around window in hen's eggs

dipped in sterile albumin, paraffin, or by means of a coverslip placed on a rim of paraffin-vaseline.¹ These methods are satisfactory when the egg is to be opened for final harvesting only, but because they are cumbersome and often lead to contamination, are impractical when subcultures and frequent smears are to be made.

These faults can be overcome by the simple expedient of attaching a sterile, cotton-plugged length of glass tubing to the egg. All manipulations thereafter are performed as with the plugged bacteriologic test tube.

* Received for publication, February 5, 1948.

MATERIALS

The materials required are the egg, glass tubing of the desired diameter and length, cotton plugs, an adhesive that will attach the glass tubing to the shell of the egg and a sterile applicator. If glass tubing of desired diameter is not available, 4 cm. lengths of glass tubing, filed from a bacteriologic test tube, may be used. The glass tubings are plugged at both ends and sterilized in the autoclave.

For adhesive material, viscoloid in acetone, Murrayite Cement (Standard Scientific Supply Corp., New York, New York, and Adams Sealing Cement (No. A6350 Clay-Adams Co., New York, New York) have been found satisfactory

The viscoloid is prepared by dissolving enough strips of viscoloid in acetone to produce a viscosity approaching that of thick molasses. The Murrayite Cement, as obtained from the manufacturer, is too thin and must be concentrated by heating and evaporation. The Adams Sealing Cement, when melted by heat, is ready for immediate use. This preparation gave the best results. It hardens within a few seconds, is easily applied and requires the least preparation.

METHOD

The egg is prepared in the usual manner and is inoculated. Using aseptic technic, the plug from one end of the glass tubing is removed, and the tube is dipped into the adhesive material to a depth of about 0.5 cm. As the tube is withdrawn, a film of the viscous material may form across the mouth of the tube. This film is easily broken with the sterile applicator. With gentle pressure the tube is attached to the egg over the opening in the shell. To insure absolute sealing more adhesive is applied with the applicator.

SUMMARY

A method is described for sealing inoculated hen's eggs with cotton-plugged glass tubing, thus converting the egg into a workable "test tube".

Acknowledgment. The photograph was made by Edward Santora, M.D., Director of Pathology, to whom I am also indebted for suggestions and advice.

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SIMPLE METHOD FOR EXSANGUINATION OF LABORATORY ANIMALS*

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The following simple method of exsanguination of rabbits and other laboratory animals has consistently yielded a maximum quantity of sterile blood.

Materials. The materials used consist of a 250 cc. sterile vacuum bottle with a rubber perforable cap of the type used in blood banks for collection of blood,

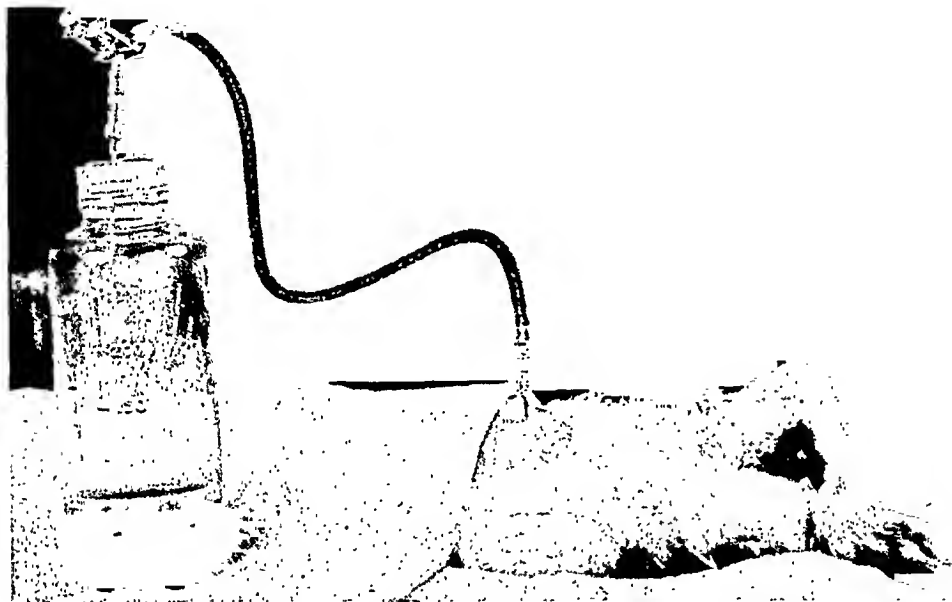


FIG. 1. Collection of sterile blood from heart of rabbit. Animal and apparatus in position.

and a 14 inch length of rubber tubing fitted with needle adapters at each end. To one adapter is attached a 2-inch long, 15-gauge needle for penetrating the cap of the bottle, while a 3-inch long, 19-gauge needle is attached to the other end for the cardiac puncture. The set is sterilized in the autoclave.

Procedure. The animal is anesthetized by an intravenous injection of nembutal (approximately 1.5 cc. for a 7 pound rabbit) and fixed to the table in the usual position for a cardiac puncture (Fig. 1). The fur over the precordium is clipped or shaved and the area painted with 3½ per cent tincture of iodine. The rubber cap of the sterile vacuum bottle also is iodinated. A screw clamp is placed over the rubber tubing and screwed down to compress the tubing completely. The bottle is then entered with the shorter needle. The apex of the

* Received for publication, February 25, 1948. This study was supported by a grant from the Tillie Bloom Fellowship Fund.

animal's heart is located by palpation, and the needle is inserted in the interspace overlying the apex and is directed toward the heart. The needle is advanced until the tip is felt against the ventricular wall. At this point the screw clamp is released, and with a sharp thrust the heart is entered. Immediately thereafter, blood may be seen entering the flask. The needle then oscillates with the contractions of the heart. Approximately three to five minutes are sufficient for the complete exsanguination of a 7 pound rabbit, from which a yield of 100 cc. may be expected. As long as there is any venous blood returning to the heart, the flow of blood into the bottle will continue. By this method, one avoids contamination of the blood and the mechanical difficulty that is often encountered when using multiple syringes.

ELECTROLYTIC DECALCIFICATION OF BONE. PRACTICAL CIRCUITS*

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A method for rapid decalcification of bone, using direct current from a battery or a rectifier and rheostat with 110 volts A. C., has been described recently.¹ Excellent results have been obtained at two hospitals, one of which is supplied

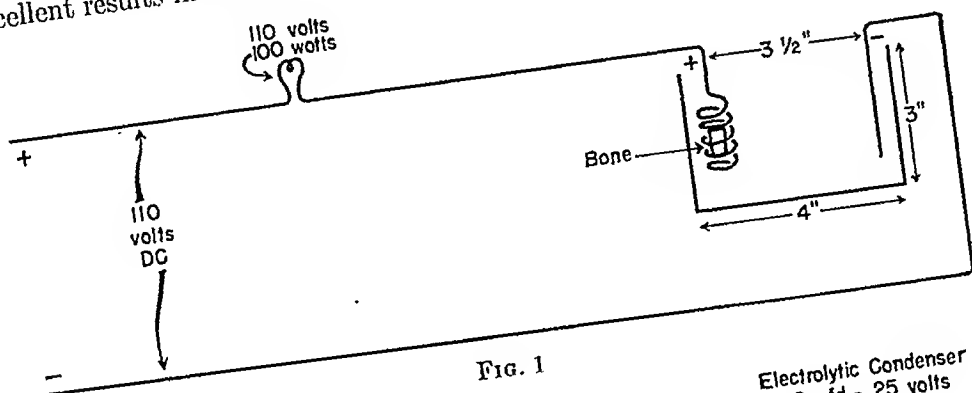


FIG. 1

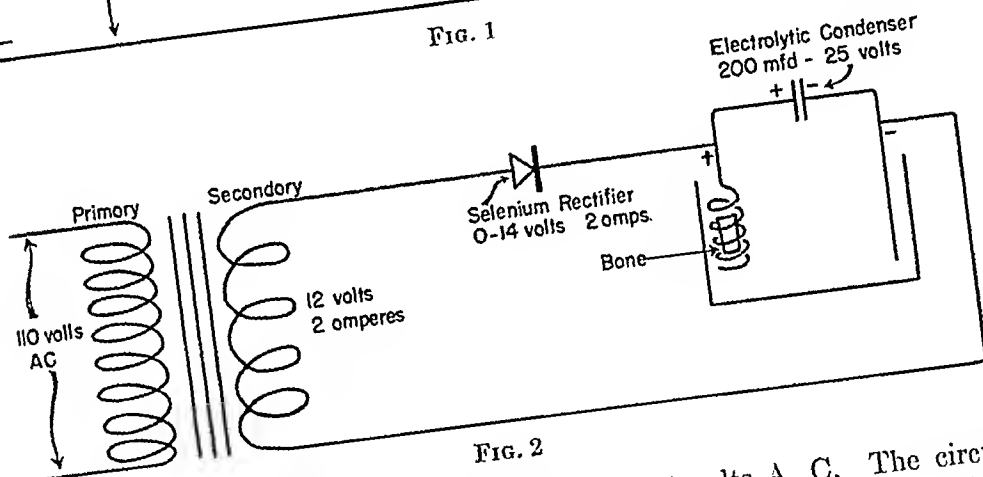


FIG. 2

with 105-115 volts D. C., the other with 105-115 volts A. C. The circuits used are detailed in the accompanying diagrams and may be easily and inexpensively reproduced.

For 110 volts D. C., a 100 watt lamp is connected in series with the electrodes (Fig. 1). The positive side of the line should be connected to an insulated lamp terminal to eliminate shock hazard and the plug marked so that it is always inserted in the outlet with the same polarity. The solution is similar to that specified by Richman, Gelfand and Hill:

Formic acid, 25 per cent	176 ml.
Hydrochloric acid, 38 per cent	40 ml.
Distilled water	284 ml.

* Received for publication, January 26, 1948.

The platinum wire is $\frac{1}{64}$ inch in diameter and the spiral coil about $\frac{1}{2}$ inch in greatest diameter. One foot of wire is required. The bone fragment is inserted tightly into the spiral and the solution placed in a glass container. No additional container has been found necessary. The electrode spacing is $3\frac{1}{2}$ inches. The voltage across the platinum electrodes is 5 or 6 volts and the current from 500 to 800 milliamperes. Under these conditions, cancellous bone is decalcified in from three-fourths to one hour and compact bone in from two to fifteen hours. Testing the consistence of the bone fragment must be done periodically with a needle or forceps to avoid over-decalcification.

For 110 volts A. C., a radio tube filament transformer furnishing 12 volts at 2 amperes is used (Fig. 2). With the same electrode spacing, wire and solutions and a half-wave selenium rectifier, capacity 0 to 14 volts, 2 amperes and a 200 mfd.-25 volt electrolytic condenser connected as detailed, the voltage and current are similar to those previously described. Values are not critical but should be uniform for best results. A decidedly superior quality of staining has been obtained in sections of bone decalcified by this method.

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SEROLOGIC FINDINGS IN PATIENTS WITH PRIMARY ATYPICAL PNEUMONIA*

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It is not possible clinically to differentiate the primary pneumonias of known viral or rickettsial etiology from the so-called primary atypical pneumonias of unknown etiology. The most important specific agents known to cause such pneumonias, namely psittacosis and Q fever, often may be suspected on epidemiologic grounds, but confirmation must come either from isolation of the agent from the patient or the demonstration of the development of specific antibodies in the blood. It is, therefore, of interest to know how frequently specific antibodies for these two known agents may develop in patients whose disease conforms clinically to the entity of primary atypical pneumonia.

The findings of specific antibodies for psittacosis and Q fever establishes a definite etiologic diagnosis for these respective diseases while the demonstration of cold hemagglutinins or streptococcus MG agglutinins, although they do not establish any specific etiology, are, nevertheless, helpful in confirming a diagnosis of primary atypical pneumonia based on clinical and x-ray findings since they are found in the majority of patients with this disease entity.¹² This report presents the results obtained using these tests on serums of 89 patients admitted to the medical wards of the Boston City Hospital, whose clinical courses, physical examinations and roentgenologic findings were characteristic of primary atypical pneumonia. The findings in most of these persons were included in a previous study,⁹ and all of these patients were seen during the period from September 1942 to March 1944. Each type of test was not done on every patient, but specimens from representative patients with different severity of the disease were subjected to each type of test. Tests for antibodies for the psittacosis group of viruses were done on the serums of all of the patients, and cold agglutinin tests were carried out on serums from all but three. The results of similar tests on 5 additional patients, having primary atypical pneumonia associated with erythema multiforme exudativum, are also included; three of them have been described elsewhere⁷ while two were seen in 1947.

MATERIALS AND METHODS

At least two serial specimens were tested on the patients studied unless otherwise noted, and a number of different tests were carried out on each specimen. Cold hemagglutination tests were performed as described elsewhere,⁸ and only titers greater than 1:20 were considered positive. Streptococcus MG agglutinin titers, determined by methods which were also described elsewhere,¹⁰ were con-

* Received for publication, April 17, 1948.

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sidered significant only if they were greater than 1:10. The complement-fixation tests for psittacosis were carried out with the 6bc strain by Dr. K. F. Meyer,¹³ those for meningopneumonitis by Dr. Thomas Francis, Jr.,¹¹ and for Q fever by Dr. Ida A. Bengston¹ using the Dyer strain. In these complement-fixation tests only a four-fold rise in titer or a titer of 1:16 (4 plus) with a subsequent rise was considered diagnostic.

Results of serologic tests. A summary of the results of the serologic tests is presented in Table 1. The data show that about 80 per cent of the patients developed cold hemagglutinins and agglutinins for the MG streptococcus. Results of the serologic tests with psittacosis and meningopneumonitis antigens indicate that only 4 of 79 patients (5 per cent) had a disease caused by a virus of the psittacosis group. Three of these patients were tested only for psittacosis anti-

TABLE 1

SUMMARY OF RESULTS OF SEROLOGIC TESTS IN PRIMARY ATYPICAL PNEUMONIA

SEROLOGIC TEST	PRIMARY ATYPICAL PNEUMONIA*		ERYTHEMA MULTIFORME EXUDATIVUM AND ATYPICAL PNEUMONIA†	
	Patients tested	Number positive	Patients tested	Number positive
Cold hemagglutinins.....	86	74	5	5
Streptococcus MG agglutinins.....	44	35	1	1
Psittacosis group‡				
Psittacosis (6bc strain).....	79	4	5	2
Meningopneumonitis.....	25	1	—	—
Q fever‡.....	25	0	3	0

* 89 cases. Severity of illness: +, 12; ++, 46; +++, 23; +++, 8.

† 5 cases. Severity of illness: ++, 1; +++, 4.

‡ Complement-fixation tests.

bodies, while one gave positive tests with psittacosis and meningopneumonitis antigens. Of these four patients, the serums of three were tested for cold agglutinins and only one was positive. Evidence for infection with the rickettsiae of Q fever was not found in any of the 25 patients tested.

The 5 patients with erythema multiforme exudativum associated with atypical pneumonia all had positive cold hemagglutination tests, while two of them also gave serologic evidence of infection with psittacosis virus.

Effect of exposure to birds. In taking the histories of all of these patients, particular attention was given to the question of exposure to birds, particularly sick birds, during the period preceding the onset of the illness. A history of recent close contact with birds was obtained in 10 of the patients with primary atypical pneumonia. None of these patients had unequivocal evidence of infection with psittacosis virus as seen in Table 2. Three of the patients raised chickens while the remaining 7 had recent intimate contact with pigeons, and two of the latter had noted specifically that the pigeons appeared sick. In one of the latter a single serum specimen taken on the seventeenth day of illness

showed a complement-fixation titer of 1:16 (4 plus) with psittacosis antigen, while in the other, serial specimens showed a suggestive increase in psittacosis antibodies; but, the results were considered inconclusive because the convalescent serum was anticomplementary in the lower dilutions. In addition to these 10 patients one of the two patients with erythema multiforme exudativum and atypical pneumonia who developed psittacosis antibodies, gave a history of

TABLE 2
RESULTS OF SEROLOGIC TESTS IN RELATION TO EXPOSURE TO BIRDS*

CASE NO.	SEVERITY OF DISEASE	COLD AG-GLUTININS	STREPTOCOCCUS MG AGGLUTININS	PSITTACOSIS GROUP COMPLEMENT-FIXATION		Q FEVER COMPLEMENT-FIXATION	HISTORY OF EXPOSURE TO BIRDS
				Psittacosis	Meningo-pneumonitis		
1	++	+	—	0	—	—	Raised chickens
2	++	+	—	+?(a)	—	—	Handled sick pigeon
3	++	+	—	+?(b)	0	0	Fed pigeons
4	++	0	—	+(c)	—	0	Handled sick pigeon
5	+	+	+	0	—	—	Raised pigeons
6	++	+	—	0	—	—	Fed pigeons
7	++	+	+	0	0	—	Fed pigeons
8	+	+	+	0	0	—	Fed pigeons
9	++++	0	—	0	0	0	Raised chickens
10	++++	+	—	+	+?(d)	0	Handled dead pigeon

* These patients had primary atypical pneumonia with the exception of Case 10 in which the patient had erythema multiforme exudativum and atypical pneumonia.

Note: None of these cases is included in the group positive for psittacosis in Table 1 since none of them meets the criteria selected for a positive serologic diagnosis. Two additional patients with atypical pneumonia and a serologic diagnosis of psittacosis (CF titers 1:256) were observed in July and August 1942; one had a single heavy exposure in a pigeon loft, and the other had played with chickens and with carrier pigeons. They are not included in this table because no cold agglutination or streptococcus MG agglutination tests were made on their serums.

Key: 0 = negative; + = positive; ? = questionable; — = not done. (a) Showed a rise in titer but serum was anticomplementary in lower dilutions; (b) single specimen taken on eleventh day had a titer of 1:16 (4 plus); (c) single specimen taken on seventeenth day had a titer of 1:16 (4 plus); (d) only one late specimen tested and titer was 1:256.

handling a dead pigeon about two weeks before the onset of symptoms.

DISCUSSION

A comparison of the occurrence of positive reactions in the various serologic tests obtained in this group of patients with the results obtained in other series of patients with atypical pneumonia reveals that the usual over-all incidence of cold hemagglutinins and streptococcus MG agglutinins is 56.7 per cent and 48.9 per cent, respectively,¹² which is considerably lower than that found here. The

observation that positive results in both of these tests are more often found in severe cases would not account for the discrepancy since most of the patients in this study had an illness of average severity.

In previous studies wide discrepancies have been observed in the percentage of patients with atypical pneumonia in whom the development of psittacosis antibodies could be demonstrated. Studies on two groups of patients^{3, 5} did not reveal any definite instance of infection with psittacosis virus, while two other investigators discovered an incidence of 8.5 per cent⁶ and 22 per cent,¹⁴ respectively. In general it has been assumed that the incidence of psittacosis among patients with the picture of atypical pneumonia was rare, but the discovery of four patients having definite serologic evidence of infection with psittacosis virus in this series of 89 patients with atypical pneumonia shows that this disease does occur at least occasionally among patients admitted to the medical wards of a large municipal hospital.

The observation that all of the patients with erythema multiforme exudativum and atypical pneumonia had cold hemagglutinins may be due in part to the fact that they were all moderately or severely ill. These results conform to those reported in four other instances of erythema multiforme exudativum² in which two patients with symptoms and signs of atypical pneumonia showed cold agglutinins, while those without pneumonia did not. The significance of the antibodies for psittacosis virus in two patients of the group reported here is not clear⁷ except that one of them gave a definite history of handling a dead pigeon about two weeks before she became ill.

In the patients studied here the history of contact with sick pigeons appeared to be a significant observation with regard to infection with a virus of the psittacosis group, while exposure to healthy pigeons or chickens did not appear to be so important.

SUMMARY AND CONCLUSIONS

In a study of 89 patients with primary atypical pneumonia a large majority was found to develop cold hemagglutinins and streptococcus MG agglutinins. Four of them developed antibodies for a virus of the psittacosis group indicating an infection with one of these agents. No instance of Q fever was found in the 25 patients whose serums were tested for antibodies against this agent. In 5 additional patients who had atypical pneumonia associated with erythema multiforme exudativum, cold hemagglutinins were present, and two of these patients showed a rise in titer in complement-fixation tests for psittacosis. A history of exposure to sick pigeons was a significant observation with respect to the development of psittacosis antibodies.

Acknowledgments. The assistance of Mildred W. Barnes in carrying out the agglutination tests is gratefully acknowledged. We are also indebted to Dr. Karl F. Meyer of the George Williams Hooper Foundation of the University of California for the psittacosis complement-fixation tests; to Dr. Thomas Francis, Jr. of the School of Public Health, University of Michigan, for similar tests with his meningopneumonitis strain; and to Dr. Ida A. Bengtson of the National Institute of Health for tests with the Q fever antigen.

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ISOIMMUNIZATION TO THE Rh ANTIGEN E IN A PERSON WITH GENES CDe*

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British Columbia, Canada*

Theoretically, immunity to a specific antigen of the Rh-complex should be produced when that antigen is introduced into the circulation of a person in whom it is lacking. The frequency of occurrence, however, of such specific isoimmunization varies greatly with the stimulating antigen. While this may be explained, in part, as due to the relative frequencies in which the various Rh antigens occur, there would appear to be a markedly variable susceptibility of the individual person to antigenic stimuli, as well as of the antigenic properties of the various Rh antigens.

The D (Rh₀) antigen most frequently evokes an antibody response and is stated to be implicated in 98 per cent of all Rh-isoimmunizations. Combinations of anti-C + D (Rh'₀) and anti-D + E (Rh''₀) also occur but with considerably less frequency. Anti-C (Rh') and particularly anti-E (Rh'') are rarely encountered alone. Of the remaining antibodies, anti-c (Hr') occurs most frequently. Anti-e (Hr'') and anti-d (Hr₀) have been reported once each by Mourant¹⁰ and Diamond,⁸ respectively.

Occurrence of anti-E (Rh'') agglutinin alone is quite rare, probably accounting for less than 0.2 per cent of all Rh antibodies. When this occurs in an Rh-positive individual, it warrants recording.

REPORT OF CASE

A 29 year old primigravida was delivered of a 7½ pound full-term male infant in the Vancouver General Hospital. She gave no history of previous transfusions or injections of blood. The course of the pregnancy was uneventful until the seventh month when a threatened miscarriage (with severe hemorrhage and uterine contractions) was averted. The patient came to term without further mishap. Twenty-four hours after its birth, the baby became severely jaundiced with other clinical manifestations of erythroblastosis fetalis.

Hematologic examination revealed that the mother, father and infant were all group A, Rh-positive (D-positive). The mother's serum, however, agglutinated both the infant's and father's cells; and the infant's cells, after repeated washings with physiologic saline, were agglutinated by antihuman globulin reagent (Coombs' test), indicating an *in vivo* sensitization of these cells. Maternal isoimmunization to an antigen in the infant's cells was postulated and a replacement transfusion undertaken. With an 18-gauge Diamond plastic catheter inserted in the umbilical vein, 180 cc. of compatible Rh-negative blood (negative to anti-C, anti-D and anti-E serums) was administered while 160 cc. of blood was withdrawn from the infant in 20 cc. amounts. The transfusion was discontinued at this point due to obstruction of the cannula. An estimated dilution of 54 per cent Rh-negative blood was achieved. Subsequently, the jaundice gradually faded. Because of a persisting, moderate anemia, a supplementary transfusion of Rh-negative compatible blood was

* Received for publication, April 19, 1948.

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given four days later. The child developed a transient spasticity which disappeared in a few days, but otherwise, an apparently good recovery was made. The significance of the spasticity has not been completely assessed, but was possibly the result of hypocalcemia produced by the citrated blood.

In view of the unusual nature of the case, specimens of blood from both parents and the child were thoroughly investigated with the available test serums. The results of these tests are tabulated below.

TECHNIC

All serologic tests were carried out using the tube technic as recommended by the Medical Research Council of Great Britain. Isotonic saline in serial dilu-

TABLE 1

	ABO GROUP	RH ANTISERUMS			
		Anti-C	Anti-D	Anti-E	Anti-c
Mother's cells.	A	+	+	—	—
Father's cells.	A	+	+	+	+
Infant's cells.	A	+	+	+	+
Maternal grandmother.	A	+	+	—	—
Maternal grandfather.	O	+	+	—	—

TABLE 2

	GENOTYPES		
	Possible	Expected frequency (Fisher)	Probable
		<i>per cent</i>	
Mother	CDe/CDe	19.00	CDe/CDe
	CDe/Cde	0.70	
Father }	CDe/cDE	10.53	CDe/cDE
Infant }	Cde/eDE	0.21	
	CDe/cdE	1.46	

tions and 20 per cent bovine albumin (Armour) in serial dilutions were used as diluents in titrations (Table 3). Tubes were incubated at 37 C. for one and one-half hours before reading. All macroscopically negative results were checked microscopically.

The essential antigenic difference in maternal and infant's cells is illustrated in Table 1 without reference to the probability of genotype structure. Infant's and father's cells were agglutinated by anti-E (Rh⁺) and anti-c (Hr⁺) serums while the maternal cells were not. It is, therefore, theoretically possible that the mother may become immunized to either the E (Rh⁺) and/or the c antigen present on her infant's cells and lacking on her own. The mother's serum was next tested against a panel of cells of known antigenic specificity. Tests were

made in serial dilutions in isotonic saline and 20 per cent bovine albumin (Armour). That the maternal antiserum contained pure anti-E agglutinins is illustrated in Table 3.

DISCUSSION

Three previous reports of anti-E agglutinin occurring alone have been encountered. One, reported by Race, was an Rh-positive woman of genotype CDe/cde (Rh₁rh) whose husband was also Rh-positive, having genotype cDE/cde (Rh₂rh). The mother developed an agglutinin of pure anti-E (Rh'') specificity. The second instance, reported by Dick,⁹ occurred in the first pregnancy of an Rh-negative woman having genotype cde/cde (rh rh); her husband had the

TABLE 3

REACTION OF MATERNAL SERUM WITH VARIETY OF ERYTHROCYTIC ANTIGENS*

ANTIGEN CONTENT OF TEST CELLS	ISOTONIC SALINE					20 PER CENT BOVINE ALBUMIN (ARMOUR)				
	1:1	1:2	1:4	1:8	1:16	1:1	1:2	1:4	1:8	1:16
A-CDe/CDe mother's own cells..	-	-	-	-	-	-	-	-	-	-
A-CDe/cDE father's cells.....	++	++	-	-	-	Not tested				
A-CDe/cDE infant's cells.....	++	+	+	-	-	Not tested				
O-CDe/cde MN-P.....	-	-	-	-	-	-	-	-	-	-
O-CDe/cDE MN-P.....	++	-	-	-	-	++	++	W	W	-
O-cDE/cde MN-P.....	W	-	-	-	-	+	+	-	-	-
O-cde/cde MN-P.....	-	-	-	-	-	-	-	-	-	-
O-cdE/cde.....	+	+	-	-	-	+	+	-	-	-
O-Cde/cde.....	-	-	-	-	-	-	-	-	-	-
O-cDe/cDe MN-P.....	-	-	-	-	-	-	-	-	-	-

* ++ indicates very large clumps under the microscope, + smaller clumps, W weak reaction with uniform distribution of small clumps of 4 to 6 cells.

genotype cdE/cde (Rh''rh). The third report, by Van Loghem,¹¹ concerned an Rh-negative male volunteer, genotype cde/cde (rh rh), who received a series of injections of blood twice weekly from a donor with genotype cdE/cde (Rh''rh). Anti-E agglutinin was produced after a course of 17 injections during a period which included a rest of six weeks.

It is apparent that there is considerable variation in individual response to the antigens of the Rh-complex. When the susceptible person is Rh-positive, immunity may be produced to other components of the Rh-complex, as occurred in the case reported by Race and in the case here recorded. In our patient, immunity was produced to only one of two possible Rh antigens after the minimum stimulus of a single pregnancy. This variation could be due to variable antigenicity of the two particular antigens contained in the fetal cells (E and c) or to specific susceptibility to the particular antigen (E) on the part of the mother.

SUMMARY

Isoimmunization to the E-antigen is reported in an Rh-positive primiparous woman. Reports of erythroblastosis fetalis which implicate specific antibodies of other antigen systems than the Rh-complex are encountered from time to time. The possibility of occurrence of isoimmunization to other antigens than D in Rh-positive individuals should be considered in assessing such reports.

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MALIGNANT MELANOMA OF THE SKIN

CLINICAL AND PATHOLOGIC ANALYSIS OF 75 CASES*

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This study concerns the clinical and pathologic features of 75 cases of malignant melanoma of the skin, of which 44 were in men and 31 in women. It does not include malignant melanoma of the oral cavity, eye, vulva or anus. Table 1 shows the distribution of these cases by age (decades) and sex.

Table 2 illustrates the high number of these lesions which occurs in the lower extremities and in the region of the head and neck.

Forty-six cases (61 per cent) had a pre-existing mole. A mole preceded the development of tumor most frequently in the lower extremities. In 15 of the 46 cases which presented a previous mole, the mole had been present since birth.

GROSS PATHOLOGY

In early malignant melanoma of the skin, evidence of origin from a previously existing mole may at times be seen (Fig. 1C). As a general rule, a malignant melanoma is elevated and frequently superficial ulceration and pigmentation are present. In a few instances, the tumor is grossly nonpigmented; but when pigmentation is present, it may be confined just to the center of the lesion, may spread to involve the entire tumor, or extend beyond to produce a sooty halo around it. On cut-section, the pigmentation may be uniform throughout the tumor, but more often it is splotchy in its distribution and, at times, particularly in the deeper areas, pigmentation may be entirely absent. Sometimes, bluish black satellite skin nodules surround the tumor; when present, these nodules are often small (0.2 to 0.5 mm.), are soft, and usually are located in the subcutaneous tissue. In advanced cases, melanomas may become quite bulky, the area of ulceration extensive, and skin nodules innumerable. It is relatively common for deep nodules to be found rather unexpectedly beneath the tumor or at some distance from it. Blood vessel invasion is rarely noted.

In 69 of our 75 cases, pigmentation was present in the primary tumor (10 had very scanty pigmentation and 4 were sectioned twice for biopsy before pigment was demonstrated). In 1 instance pigment was not present in the primary tumor, but was present in the metastasis; in 2 instances pigment was present in the primary and not in the metastasis; and in only 5 cases was pigment not seen in either the primary tumor or its metastases.

Malignant melanomas metastasize widely, and organs usually spared by epithelial neoplasms are frequently invaded. From a total of approximately 22,000 au-

* This study was financed in part by Cancer Research Fund "B". Presented at the Twenty-Sixth Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 28, 1947. Received for publication, October 27, 1947.

topies at Washington University, St. Louis City Hospital* and the Jewish Hospital,† 12 cases of malignant melanoma of the skin were collected. The metastases in these cases were widely disseminated. In all instances, regional lymph nodes were involved, and in many instances the node involvement was massive. The lungs and liver were each involved in 9 cases. The liver was usually enlarged and in 2 instances weighed over 5000 Gm. The heart was involved in 7 subjects. Such involvement is common and frequently the pericardium, epicardium and

TABLE 1
DISTRIBUTION OF PATIENTS ACCORDING TO SEX AND AGE IN DECADES

AGE	MEN	WOMEN
10-19	0	1
20-29	2	1
30-39	1	5
40-49	4	5
50-59	7	6
60-69	13	5
70-79	13	7
80-89	3	1
90-99	1	0
Total.....	44	31

TABLE 2
LOCATION OF LESIONS

LOCATION OF LESIONS	NUMBER	PER CENT	PREVIOUS MOLE*
Lower extremities.....	26	35	21
Head and neck.....	24	32	10
Chest.....	16	21	10
Upper extremities.....	7	9	4
Trunk.....	2	3	1

* The word mole is used in preference to nevus since nevus is a nonspecific term used for a multitude of skin conditions.

myocardium are included.³⁹ The skin and the intestinal tract (usually the submucosa) each presented metastatic lesions in 6 cases. In the intestinal tract, the ileum was most commonly involved and the large bowel was affected in only 1 case. The kidneys and spleen were each involved in 5 cases, the adrenals in 4, thyroid in 4 and bone in 4. In one instance, involvement of ribs and vertebrae was so extensive that a clinical diagnosis of multiple myeloma was entertained. Many other organs, including pituitary and ovary, were involved in single cases.

* Material made available through courtesy of Dr. John Saxton.

† Material made available through courtesy of Dr. Sam Gray.

MICROSCOPIC PATHOLOGY

Benign moles are often erroneously considered malignant because they are non-encapsulated and extend deeply into the subcutaneous tissues. Individual cells of these moles, however, are uniform in size and shape, without mitotic figures. Very cellular moles are confusing, but these tumors are recognized by typical configuration rather than by their cellular detail and the cells are often arranged in nests, bands, or strands. The presence of nerve-end organs or caricatures of them add to the difficulty of diagnosis (Fig. 3).

Mole cells are rarely epidermal, being located entirely in the epidermis. Most commonly, however, these cells are intradermal being located entirely within the dermis. Fairly frequently, mole cells lie against the epidermis, or are seemingly within it, and the tumor is called a junction-type mole. Ewing¹² reported 2 melanocarcinomas in which the tumor cells were entirely within the epidermis. Traub and Keil⁴⁴ believe that melanocarcinomas only rarely arise from epidermal moles, and further, that the common intradermal mole is rarely the point of departure for melanocarcinoma. They stated that they had never seen an intradermal nevus on the lower extremity where melanocarcinomas are commonest. A search at this hospital and at two large metropolitan hospitals revealed 4 intradermal moles of the thigh but none below the knee. All those below the knee were of the junction-type. Unfortunately, in all but 3 of our melanocarcinomas the neoplasm was so far advanced that any guess as to the type of mole from which it arose would have been pure conjecture. In these three instances the moles were of the junction-type. Stout³⁹ has seen an early melanocarcinoma arising from an intradermal mole. Nicolau³¹ found the upper extension of intradermal metastases to involve the epidermis and drew parallelisms between this and primary melanocarcinomas. It would be pertinent for pathologists who see early melanocarcinomas to make serial or step sections in an effort to determine whether the junction-type moles are the dangerous ones. It seems certain that other types (epidermal and dermal) are also points of departure for this malignant tumor. The clinical types of potentially malignant moles seem much more firmly established than the microscopic types.

The earliest malignant change in moles usually occurs in a group of cells which frequently show enlargement of both cytoplasm and nucleus. The nuclear changes are most important; the nuclei may become bizarre with mitotic figures and nucleoli are often prominent. The rete columns may be enlarged and replaced by tumor cells and chronic inflammation is often present in the dermis.⁹ Borderline changes in moles may be difficult to evaluate, and at times, multiple sections may be necessary. Infrequently, tumor cells are observed in the lymphatics of the skin. It is most unusual to find the changes of malignant melanoma entirely within the epidermis with no change in the dermis.¹² It should be remembered that malignant changes may appear to develop in a prepubertal child but these changes, in this age group, are usually not clinically significant.

The microscopic appearance of a malignant melanoma is extremely variable. If large amounts of melanin pigment are present, then the variability is seldom confusing. Practically all melanocarcinomas are pigmented to a certain extent,



FIG. 1A. Classic pigmented ulcerated melanocarcinoma of preauricular area; B. Intensely pigmented malignant melanoma arising from previous soft brown mole. To the left are two satellite nodules; C. Malignant melanoma of preauricular area arising from a previously existing mole. Note other pigmented areas. (Courtesy C. V. Mosby Company, 1947); D. Melanocarcinoma of temporal region after a full therapeutic course of irradiation. Note persistence of the tumor.

but it must be stressed that the amount of pigment may be very scanty, may not be present in all sections, and perhaps may be seen within only a few cells. The typical microscopic changes are more frequently present in areas of transition between normal and involved epidermis. Sections from this zone often determine

the pathologic diagnosis. A biopsy from a centrally ulcerated melanoma may contain no pigment. We had 3 melanocarcinomas of the skin in which superficial biopsies revealed no pigment but which on cut-section showed large amounts of pigment present in deeper areas.

The recognition of pigment as melanin is usually not difficult, but it must be differentiated from hemosiderin, which is often refractile, has large granules of golden yellow color and takes a specific stain for iron. Hemosiderin is also frequently, but secondarily, associated with melanocarcinoma because this tumor is rapidly growing and prone to hemorrhage. Melanin, by contrast, is brown, at times has a faintly greenish tinge, is finely granular, is not refractile and does not take the stain for hemosiderin. The presence of small amounts of melanin is often enhanced by the use of silver stains, such as Fontana's, which may be of diagnostic value.

The greatest difficulty in microscopic diagnosis arises with the uncommon non-pigmented or very scantily pigmented melanocarcinoma, for any atypical or bizarre malignant skin neoplasm may be a malignant melanoma. The usual malignant melanoma, however, often shows rosette-like areas in which the arrangement of cells is very similar to the arrangement of cells in a benign nevus (Figs. 4A and 4B). On the other hand, it may mimic with considerable accuracy other malignant neoplasms,⁸ such as fibrosarcoma with long spindle-like cells which have rather prominent nuclei; but in contrast to fibrosarcoma, the melanoma often has prominent nucleoli (Fig. 5A). The cells of malignant melanoma often show a tendency to arrangement in small nests walled in by bands of connective tissue (Fig. 5C). The pattern may also suggest a very undifferentiated carcinoma with round cells, inconspicuous cytoplasm and even fine nucleoli (Fig. 5B). In many malignant melanomas, vacuolated areas may appear, particularly in the cytoplasm, which could suggest a neoplasm containing fat, such as a liposarcoma (Fig. 5D). One of the rarer variants is the type presenting extremely large cells suggesting ganglion cells (Fig. 6A). These cells may have multiple nuclei, usually have prominent nucleoli and an invariably acidophilic cytoplasm; such a variant perhaps supports the neuroectodermal origin of malignant melanoma. Another type resembles the spindle-cell variant of epidermoid carcinoma (Fig. 6B).

The dopa test described by Bloch⁵ can be diagnostically helpful. The melanoblast is designated as the cell which forms melanin pigment, and the chromatophores or melanophores are the cells which contain melanin pigment which they have obtained elsewhere by absorption or phagocytosis. Certain so-called dopa-oxidase cells contain the pigment-forming ferment which is capable of converting the colorless dopa to a dark insoluble melanin, the dopa melanin. This increased darkening is proportionate to the quantity and activity of the ferment and demonstrates that pigment is formed in the protoplasm and not in the nucleus. Melanoblasts, at times, have branching processes which give them the name of dendritic cells. The dopa reaction is positive in the cells of the basal layer of the epidermis and follicles and in the matrix of pigmented hairs. The dopa reaction is positive also in the cells of pigmented brown moles and in the melanocarcino-



FIG. 2A and B. Two melanocarcinomas of the plantar surfaces of the foot, showing variation of pigmentation and elevation.

mas which arise from such moles. If a tumor is suspected of being a nonpigmented melanocarcinoma, fresh tissue should be obtained and stained without delay by the substrate dopa (dioxyphephenylalanine); if melanin is present, the dopa becomes oxidized to melanin and is seen as dark pigment. A negative dopa test does not rule out a malignant melanoma. Unfortunately, when the pathologic diagnosis is uncertain, the tissue is often put in fixative and then a dopa test cannot be used. In generalized melanocarcinomatosis, melanin may at times be demonstrated in urine, probably because of breakdown of tumor cells, absorption of pigment into the blood and its excretion through the kidney. Forty-seven of our patients were tested for melanin in the urine. In 33 instances the test (ferric chloride) was negative; 16 patients were clinically free from metastases, but 8 later died of the disease. Seventeen of the negative group had definite local and, at times, distant metastases, and 14 of these died of the disease. In 14 instances, the melanin test was positive and the clinical diagnosis was obvious; in practically every one of these instances the process was generalized. Only one of these patients is living; she had a malignant melanoma of the foot. In 1 patient, the test at first positive, later became negative; this patient later developed three local recurrences. More sensitive tests have not proved practical. It should be remembered that other conditions, particularly those in which protein destruction is prominent (intestinal obstruction, acute pancreatitis), may have associated transient melanuria.¹⁸

In examining lymph nodes which have been removed in the course of dissection (neck, axilla, or inguinal regions), it is not unusual to find macrophages swollen with melanin pigment. This pigmentation may occur in spite of the absence of tumor cells in the lymph nodes, particularly when there has been a previous heavily pigmented malignant melanoma. The significance of this finding is debatable, however. Experimentally, in the transplantable malignant melanoma of the mouse reported by Harding and Passey,²⁰ axillary nodes which were heavily pigmented (due to melanin in macrophages) were transplanted into other presumably susceptible mice. These transplants grew in only one instance.

A transplantable mouse tumor (melanocarcinoma) reported by Harding and Passey²⁰ was used for further experimentation in this country by Hogeboom and Adams²³ who demonstrated that melanocarcinoma, which probably arose from the skin melanoblast, contained extractable enzymes which catalyzed the oxidation of both tyrosine and dihydroxyphenylalanine to melanin. The enzyme, tyrosinase, had not previously been shown to occur in mammalian tissue. Greenstein¹⁶ studied the oxidase activity of melanotic and amelanotic melanomas and found that the melanotic group presented tyrosine and dopa-oxidase and also, a cyanide-insensitive system which oxidized p-phenylenediamine. Activity of cytochrome oxidase in both melanotic and amelanotic melanomas was of the same order of magnitude. Sugiura,⁴¹ working with the Harding-Passey mouse melanoma, found that the age of the host did not influence the outcome of transplantation, also that castration in males and females did not affect the growth of the tumor. Viability of the melanoma was destroyed by dehydration from its frozen state and the growth itself was not filterable.

It is widely accepted that melanocarcinoma and pigmented moles have a neurogenic origin. Soldan,³⁸ in 1899, believed that moles of the skin were neurofibromata of the tactile terminations and were formed by proliferation of the connective tissue of the dermis. Masson²⁶ believed that they did not represent

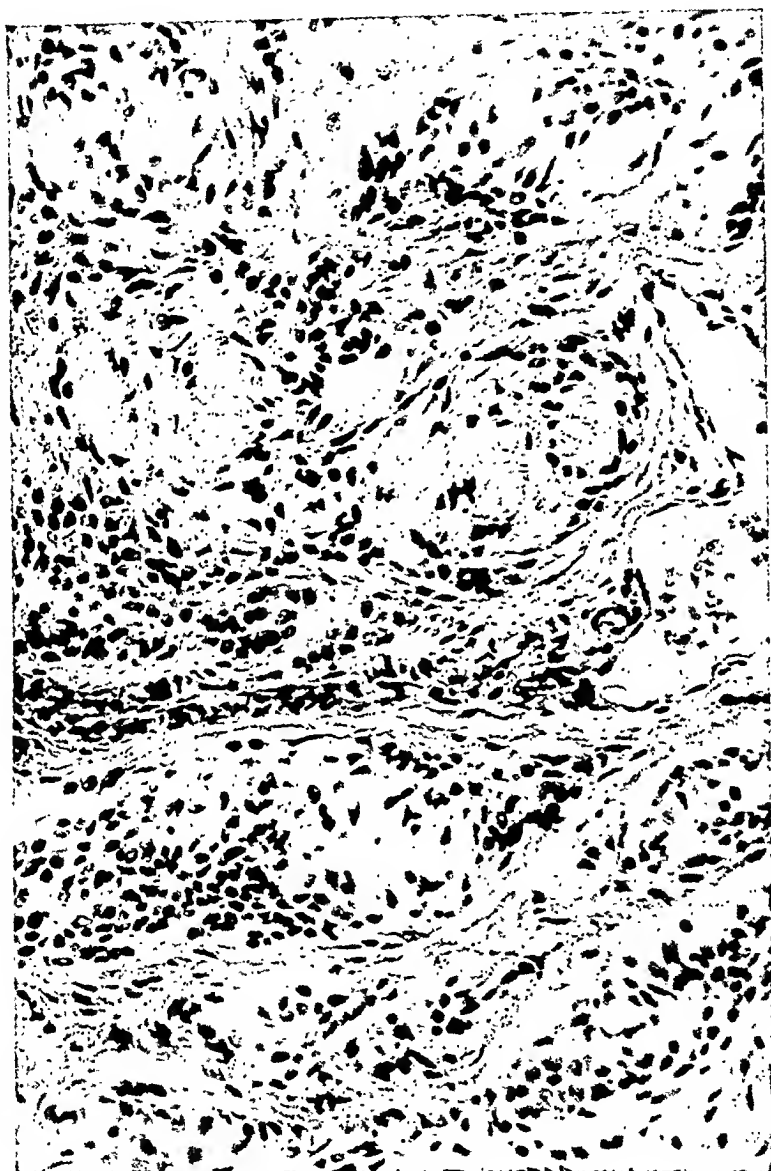


FIG. 3. Photomicrograph of benign mole showing an area in which caricatures of Wagner-Meissner bodies are present.

connective tissue tumors but were derived from the Schwannian syncytium. Masson^{26,27,28} and Ewing¹² described the tactile nature of neval nerves and Masson²⁶ was the first to describe caricatures of Meissnerian tactile corpuscles. These changes were also corroborated by Foot¹⁵ and Stout⁴⁰. Special silver staining as demonstrated by Laidlaw and Murray²⁵ and by Foot¹⁴ show that these moles are

rich in nerve fibers and tactile cells. End bulbs of nerve fibers make exact contacts with neval cells (just as they do in normal skin) and with tactile cells of the epidermis and the hair follicles. Moles resemble the tactile spots of reptiles and amphibia. Reptilian tactile spots in the course of evolution are replaced by mammalian hair follicles (cat whiskers) and, therefore, the hairy pigmented mole is apparently a transition or link from pigmented tactile organs of mammalian type. In its hair follicles it is mammalian, and in its pigmentation-elevation and in the innervated tactile cells in the corium, it follows a reptilian-amphibian character.²⁵

CLINICAL EVOLUTION

In approximately 65 per cent of the instances, malignant melanoma of the skin arises from a mole which often has been present from birth.⁴⁵ The clinical type of moles from which melanocarcinoma can arise is still debatable. In our series the commonest type was a *flat, soft, nonhairy, brown mole usually no more than 2 or 3 cm. in size*. At times the mole was brown but changed to blue, brownish black, or black. In several instances, it was black or bluish black from the beginning. It seems likely, therefore, that when a mole becomes black it is already a melanocarcinoma. How long it remains localized as such is not known. No tumor in our group was hairy; this observation has been reported by others.² In 2 instances only, it was large, hard, elevated and warty. One of these was on the foot and the other on the scalp.

A mole does not usually undergo malignant change during childhood or adolescence. The first signs of malignant change in a mole are *darkening or pigmentation, increased growth, surface ulceration and extension of the tumor into the surrounding skin* which may result in a sooty-like halo about the tumor. This sequence of events may occur without previous injury, but is sometimes related to trauma, often chronic in nature. In our group of cases, explosive development of malignant melanoma occurred in a pigmented mole of the cheek which had been subjected to daily shaving and in a pigmented mole of the posterior portion of the chest which was irritated by a mailbag strap; a malignant melanoma of the foot developed following continuous irritation by an ill-fitting shoe. It is not known how trauma to a mole occurring over a long period of time will suddenly cause a change in the lesion and the development of malignant melanoma.

Anderson⁴ reported that malignant melanomas infrequently occur in the American Negro, but emphasized the frequency with which trauma was associated with the neoplasm. In this regard, DesLigneris¹¹ and Hewer²² have stressed the frequency of this tumor among the natives of Africa. Practically all the tumors in their group appeared in the region of the leg or foot, and they were frequently related to injury, as by thorns. Recently Pack *et al.*³⁵ reported that of 862 malignant melanomas, 10 occurred in Negroes and stated that they developed more commonly in mulattoes in the oral cavity, nail bed and sole of the foot. These areas are relatively nonpigmented zones and this may be the reason for the location.

In approximately 10 per cent of the cases, the first indication of tumor may be a large metastatic node located in the axilla, cervical region, or inguinal area.

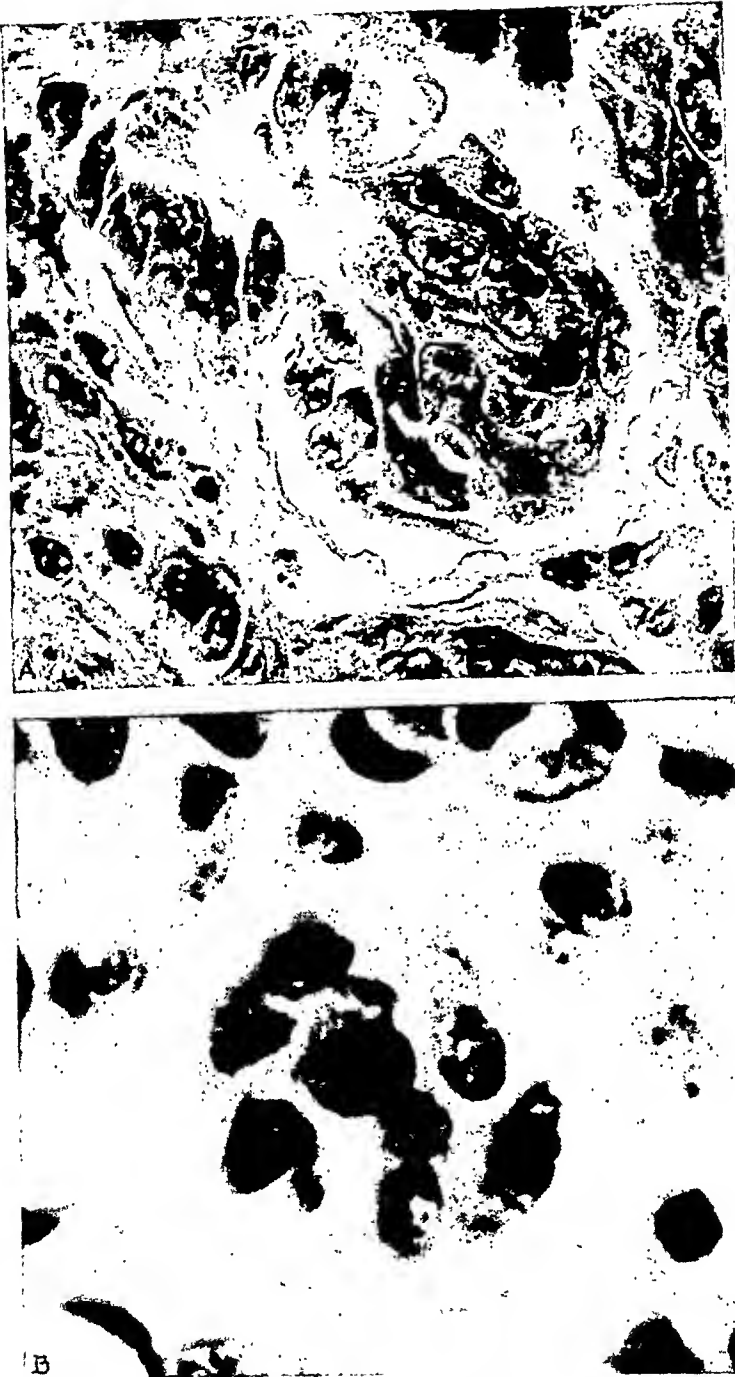


FIG. 4A. Typical arrangement of mole cells in a benign neoplasm. B. Cluster of malignant cells in a melanocarcinoma very suggestive of the arrangement of cells in Fig. 4A. High power.

Careful questioning may elicit the information that an inconspicuous, apparently innocuous, mole has been removed from a skin area which drains into the implicated node. In a small percentage of cases (perhaps 5 to 10 per cent) the first symptoms are due to liver metastasis or are those of intestinal obstruction due to

metastasis to the gastro-intestinal tract. In 3 of the autopsied cases, a primary skin lesion was not noticed or suspected. One patient was operated on for what was thought to be a brain tumor but proved to be a metastatic melanoma; a second patient entered the hospital with a large nodular liver, and a third had signs of small bowel obstruction.

If the melanoma is first seen after it has already metastasized, the evolution of the disease varies widely; usually death occurs within three years, but in some instances the entire evolution may be less than one year. In 2 of our cases the metastases developed so slowly that the patients lived five years with evidence of metastatic disease.

DIAGNOSIS

In most instances, the clinical diagnosis of malignant melanoma of the skin is relatively simple. In a high proportion of cases reported, the diagnosis was made at the time of clinical examination, but of course was easiest where the clinical course was typical. In our group, most of the patients presented a mole, which in some instances had been present since birth, and which frequently had been subjected to chronic irritation. The previously innocuous mole suddenly began to increase in size, became deeper in color and pigment often extended around it. The test for melanin in the urine was not diagnostic in our series, for when the disease was localized and the clinical diagnosis was doubtful, the melanin test was negative; even with definite metastases it sometimes continued to be negative. It was only when the disease was widespread that the test for melanin became positive. False-positive tests (using ferric chloride) can occur if the patient is taking aspirin.

In our series, malignant melanoma was easily diagnosed when the tumor was deeply pigmented and showed ulceration and bleeding (Fig. 1A). When the primary lesion was small and the metastases were deeply pigmented and visible, then of course, the tumor was again easily recognized. The diagnosis became most difficult when the course was atypical and the initial lesion relatively nonpigmented or inconspicuous. Sometimes, however, the location of a nonpigmented malignant melanoma may indicate or facilitate the correct diagnosis (plantar surface of the foot, hair line near the temple, or subungual³³ zone). In the region of the face, there were 6 lesions which were first diagnosed clinically as basal cell carcinoma because of scanty or no superficial pigmentation. In the upper extremity, 4 cases were not recognized at clinical examination because 1 of them masqueraded as a soft tissue sarcoma, another presented no demonstrable primary but numerous subcutaneous nodules, and the other 2 showed little pigmentation.

The best degree of correlation between clinical and pathologic diagnoses occurred on the trunk, where all but 1 of the tumors were deeply pigmented, and all but 2 had previously been moles; often the regional lymph nodes were implicated. In one instance, because the tumor was infected, small and inconspicuous, a diagnosis of infected papilloma was entertained.

It must be stressed that malignant melanoma is the most common tumor of

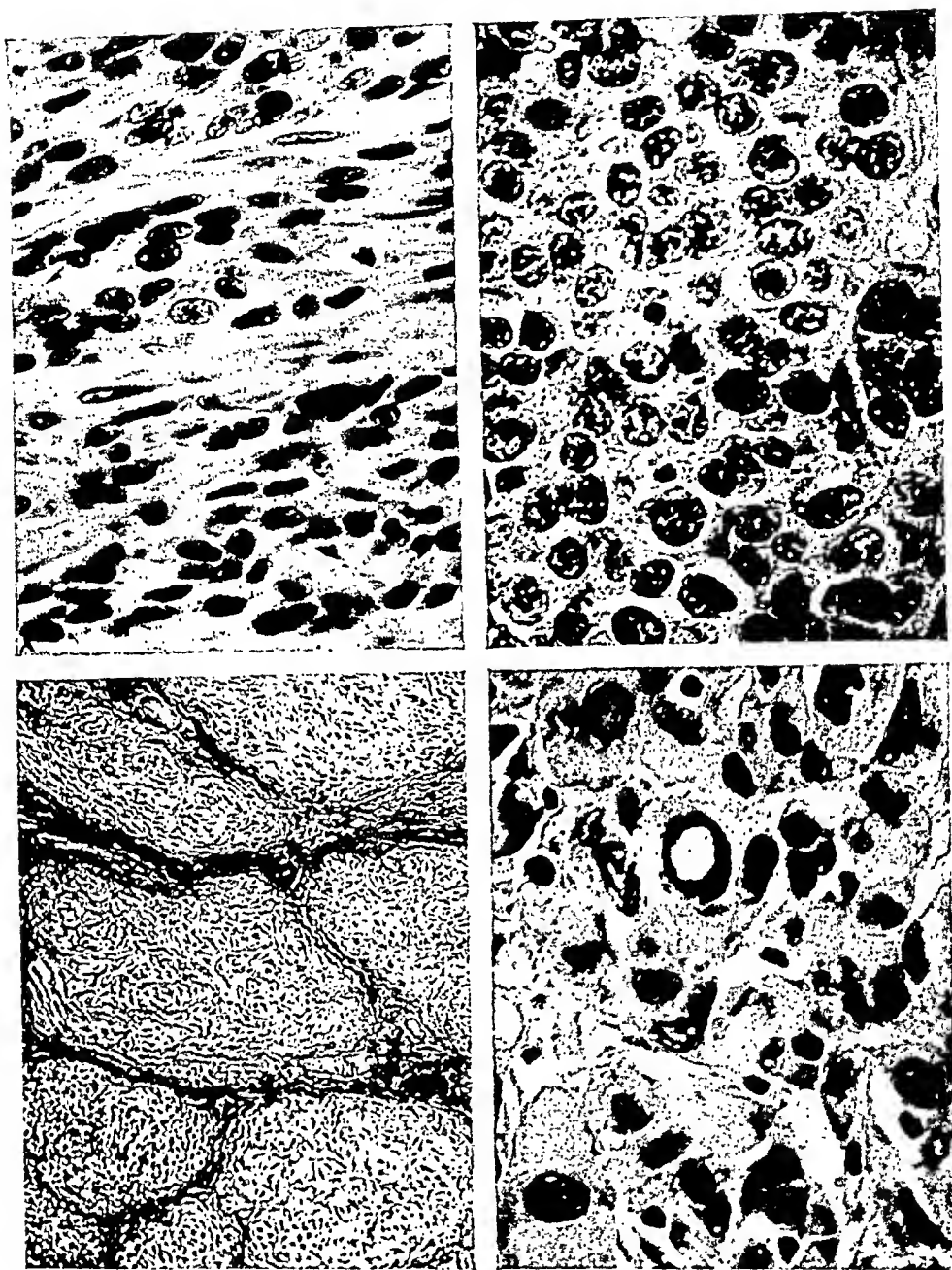


Fig. 5A. Relatively nonpigmented melanocarcinoma suggesting fibrosarcoma. B. This variant of melanocarcinoma suggests undifferentiated carcinoma. C. Wilder stain to demonstrate reticulum in a melanocarcinoma. Moderate enlargement. D. Very large cells, one of them vacuolated, which resembles a sarcoma such as a liposarcoma. High power, magnification same as A and B.

the lower extremity and is about twice as frequent as all other malignant tumors in that area. We have had 26 malignant melanomas of the lower extremity and only 14 skin carcinomas during the same period. Daland and Holmes⁷ reported that 55 per cent of their malignant lesions of the lower extremity were malignant

melanomas. In 3 cases of melanoma of the lower extremity, definite diagnoses were not made: one was thought to be a hemangioma; although another appeared on the plantar surface of the foot, it was nonpigmented; and the third patient entered with a node in the groin without a noticeable primary lesion. There were 5 others in which alternate diagnoses, such as epidermoid carcinoma or sarcoma, were considered along with melanoma.

A malignant tumor of the plantar surface of the foot should be considered a malignant melanoma until proved otherwise. At times they are confused with a plantar wart or an abscess (Figs. 2A and 2B). Other lesions, such as pigmented papillomas, hemangiomas (especially those with infection), seborrheic keratosis and pyogenic granulomas may resemble melanoma. So-called sclerosing hemangioma occurring particularly on the anterior surface of the tibia has been confused both clinically and microscopically with malignant melanoma and the large amount of hemosiderin present is confused with melanin.¹⁷

Most malignant melanomas can be diagnosed at clinical examination and radical local excision can be undertaken without delay. Where the diagnosis is doubtful, however, we do not hesitate to do careful incisional biopsies, removing a thin wedge of tissue which includes both normal as well as abnormal skin. In our experience, biopsy has never resulted in dissemination of disease. We have excised innumerable pigmented and nonpigmented moles and even in those instances where excision was incomplete, we have never seen transition of a benign mole to a melanocarcinoma.

The *blue nevus* and the *pigmented basal cell carcinoma* also contain melanin pigment. The blue nevus, however, usually can be differentiated from a malignant melanoma as it has a slate blue color and usually does not grow to a size of more than 1 cm. These tumors are thought to be mesodermal in origin and related to the Mongolian spot. Microscopically, as Montgomery²⁹ pointed out, there is no pigmentation of the overlying epidermis and there is a clear zone free from pigmentation between the epidermis and the involved dermis. The cells also have a spindle-like character and make up an interlacing disorganized network in which there are large amounts of pigment. They rarely, if ever, become malignant. The pigmented basal cell carcinoma is an infrequent neoplasm. The pigmentation varies from minimal to very large amounts, is distributed either within the cells, in large masses around the cells, or free within the central zones of the tumor; but in practically all instances, the morphologic pattern of the basal cell carcinoma is typical. Infrequently, epidermoid carcinomas can be associated with small amounts of melanin pigment. The difficulties of the microscopic diagnosis of the relatively nonpigmented melanocarcinoma have been discussed.

TREATMENT

Prophylactic treatment. Obviously all moles cannot be removed. Any mole, however, no matter what type (hairy, warty, junction), should be excised if it is located in an area where it is subjected to repeated trauma. These areas are predominantly the face (constant nicking by a razor), the neck (rubbing by a collar),

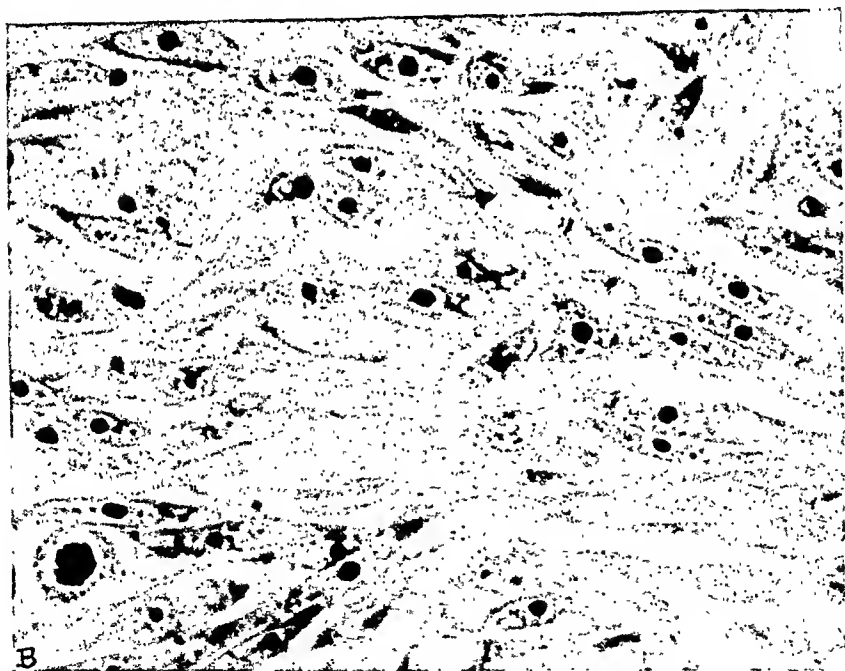
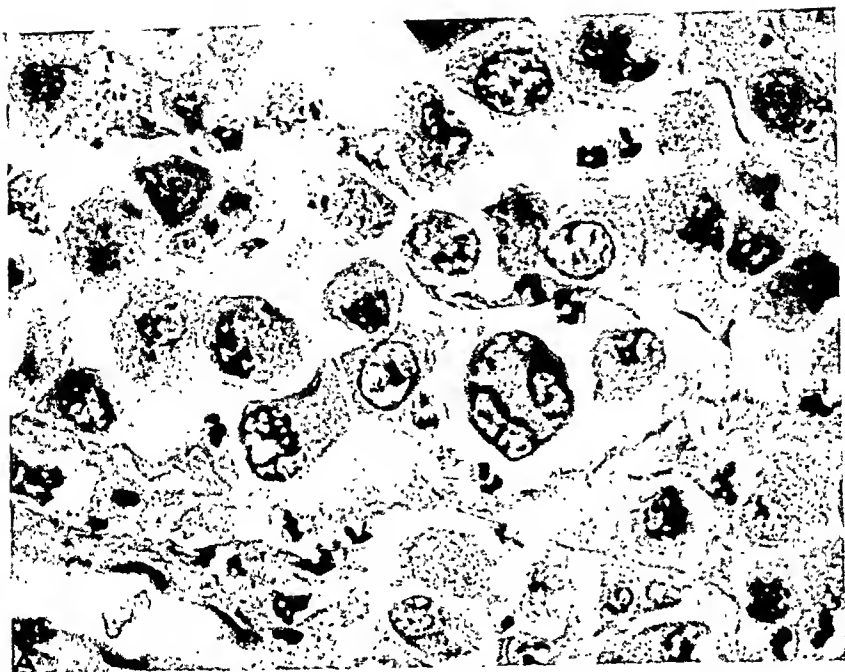


FIG. 6A. Rare variant of melanocarcinoma with huge cells, some of them multinucleated, suggesting ganglion cells. B. Common variant of melanocarcinoma with spindle-like cells and very prominent nucleoli suggesting spindle-cell variant of epidermoid carcinoma. High power. The magnification of Figs. 5 A, B and D and 6 A and B is the same.

and the chest (irritation by a strap). The moles may be located on the abdomen where they may be subject to rubbing by a belt. Any mole which shows increased growth or deepening of pigmentation should also be excised. Moles

located in areas often the site of malignant melanoma should be given particular attention. This includes the hair line near the temple, the subungual area, and particularly the lower extremities. On the lower extremities, all moles subjected to trauma as well as all soft, brown, hairless, very slightly elevated moles should be removed. In the absence of change in character and without chronic irritation, the warty mole, the hairy mole and the common soft intradermal mole do not need excision. Deeply pigmented hair-free moles in the pre-pubertal child should probably be excised. All moles should be removed by cold steel so that adequate histologic examination can be done. The use of the electric needle, carbon dioxide snow, or other methods of cauterization are to be condemned.

Castration. Because malignant melanomas have been shown to metastasize reluctantly during puberty but to increase with pregnancy, a hormonal influence should be considered.⁴⁵ Herbst²¹ undertook castration of a patient with a malignant melanoma of the choroid and obtained some temporary improvement. Howes,²⁴ on the other hand, treated a malignant melanoma of the conjunctiva in a similar fashion with absolutely no effect. The castration of both male and female mice with Harding-Passey melanoma by Sugiura⁴¹ did not affect the growth of the tumor.

Radiotherapy. Radiotherapy is not indicated as a primary therapeutic weapon for the treatment of malignant melanoma. Only by cauterizing doses can this tumor be sterilized; this lack of radiosensitivity is undoubtedly related to its neuroectodermal origin. With the idea of palliation only, 3 patients in our group received radiotherapy. The first presented a local recurrence and the other two had metastatic inguinal lymph nodes. In the latter cases, the disease was localized to a fixed group of nodes. Rather marked local regression of the lesion was seen in the patient with recurrence and in one with inguinal metastases, but in both instances this was followed by increased growth of the tumor, distant metastases and death. In two other instances because of an error in the pathologic diagnosis, a primary malignant melanoma was treated with the usual adequate radiotherapeutic dose for skin carcinoma. In the first, which was called a basal cell carcinoma, the patient had a nonpigmented melanocarcinoma of the ear, measuring 2 x 2.5 cm. which was given 300 r daily, using 200 K.V., and a total dosage of 3000 r. The infection cleared, but the size of the tumor remained the same. After excision, which took place two months following completion of therapy, the melanoma showed practically no alteration. There were skin changes suggesting fairly profound irradiation effect with absence of skin appendages, but in immediately contiguous areas, the tumor was still growing. In the second case, a nonpigmented melanocarcinoma of the temple was thought to be an atypical basal cell carcinoma and was given thorough irradiation, resulting in a prominent effect on the surrounding tissue, but the melanocarcinoma persisted in the center of the irradiated field (Figs. 1D, 7A and 7B).

In a very few instances in the literature palliation from roentgen therapy has been achieved. It is regrettable that at times, patients for whom pathologic con-

FIG. 7A. Photomicrograph of tumor depicted in Figure 1D, following irradiation. Note absence of skin appendages and atrophy of the skin. Tumor cells appear viable. B. Area from Figure 7A. This shows that the melanocarcinoma is not affected by irradiation. High power.

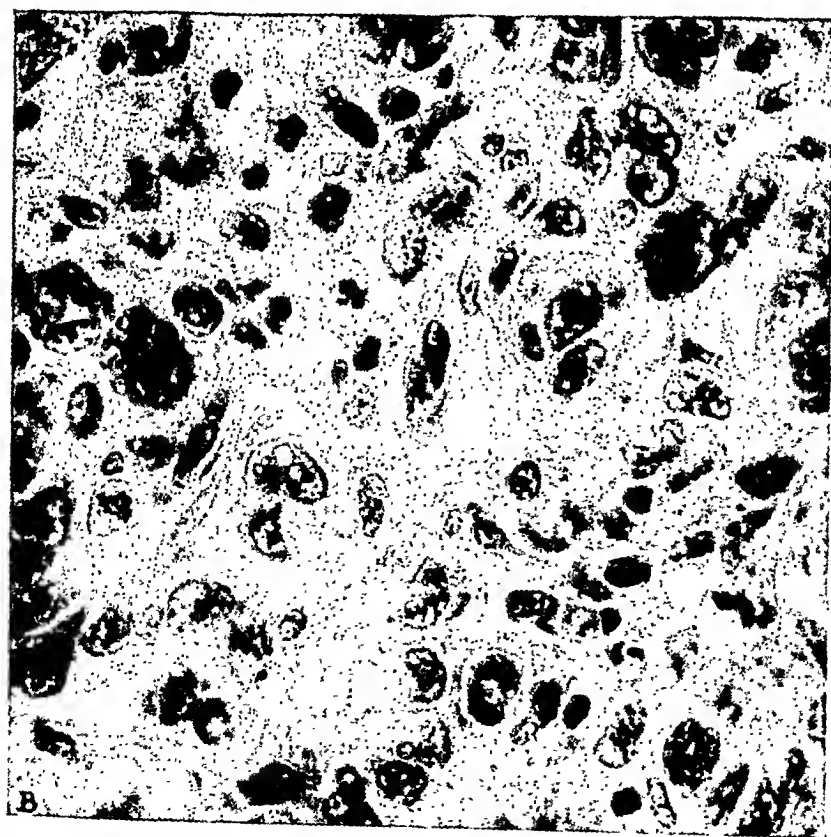
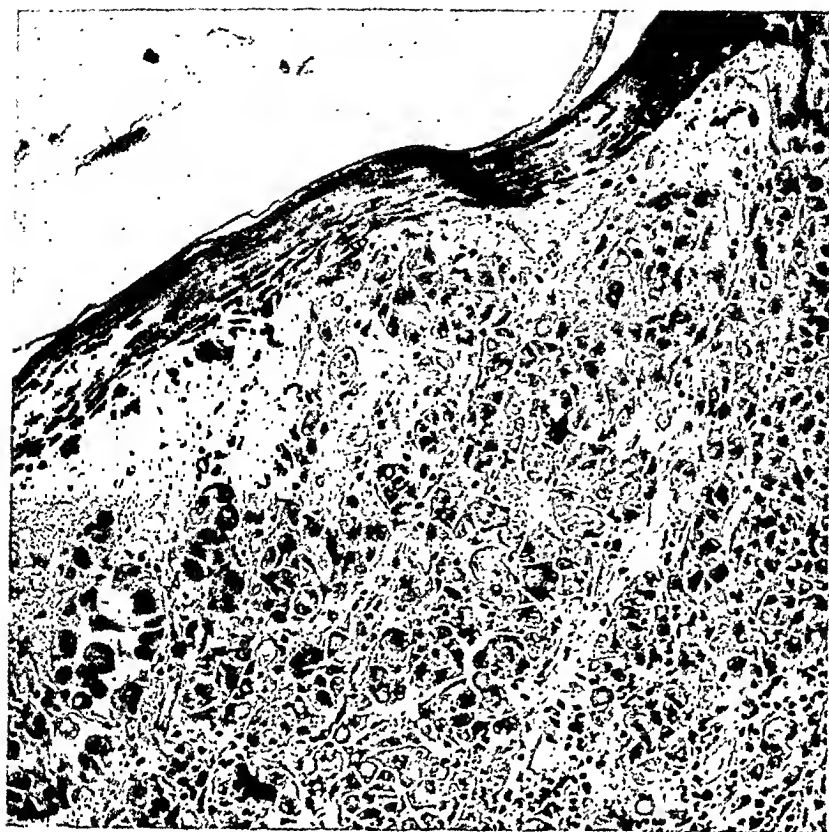


FIG. 7

firmation of the lesion has not been available, have been reported as cured by radiotherapy, for these reports are of no value. Many, if not all of these lesions, were probably benign (pigmented papilloma, infected papilloma, seborrheic wart) or radiosensitive malignant lesions (pigmented basal cell carcinoma).

SURGERY

It is unfortunate that a high percentage of malignant melanomas are far advanced when first seen, and of these, the large majority have had previous improper treatment (often including burning with a cautery, inadequate excision, freezing with carbon dioxide, removal by ligature, application of zinc chloride paste, temporizing with various local medications and the use of the electric needle). Tampering also may be done by the patient, a barber, or a beauty operator. This delay in proper treatment may cost the patient his life. The only hope of curing a malignant melanoma lies in radical surgical excision and radical dissection of the predictable or known lymph node-bearing areas. Cold steel should be used in preference to cautery, since cautery chars tissue, often renders it unsatisfactory for pathologic examination, and may leave unsightly scars necessitating plastic repair.⁴⁵ In electro-coagulation, tissue distends lymphatics and small venules which may cause tumor cells to be driven beyond the area of excision. Amadon³ reported 27 cases in which there was 100 per cent local recurrence following electrocoagulation. It has been further suggested by Handley¹⁹ and Pack³⁷ that all intervening lymphatics between the primary growth and the draining lymph nodes should be excised. We believe that in most instances the tumor spreads by emboli to the draining lymph nodes. Pack³⁷ believes that an interval of ten days to two weeks should elapse between excision and regional node dissection. This time interval is allowed for tumor cells between the primary neoplasm and the regional nodes to travel to these nodes. Otherwise, it is argued that immediate regional dissection may be followed by skin recurrences. We believe that tumor cells metastasize to regional nodes as emboli and that if the tumor does occur in the skin following a node dissection, it is due to blocked lymphatics and that such recurrences would appear regardless of the waiting period. We see no contra-indication to such procrastination, but we do not believe that the prognosis would be altered if the node dissection was done at the same time as the excision of the primary. Malignant melanoma, however, may spread locally, may grow within the dermal lymphatics, or may spread downward to the lymphatics overlying the fascia and then by retrograde extension, grow upward to form a subcutaneous nodule. It does not seem worthwhile, in most instances, to perform the radical procedures which would be necessary if Pack's principle were carried out. It would seem rational, however, for the surgical excision to be a dissection *en bloc*, with regional radical lymph node dissection if the tumor were located near the draining lymph nodes. This could be done when the melanocarcinoma is located close to the inguinal area, the cervical area, or the axilla. This principle might also be followed if satellite skin nodules exist between the tumor and the regional involved nodes (example: tumor

on foot, satellite skin nodules of leg, and involved inguinal nodes). However, if a melanocarcinoma is located, for instance, on the foot or hand without intervening skin nodules, we do not feel dissection in continuity is indicated.

When there is inguinal or femoral node involvement secondary to a malignant melanoma of the extremity, Paek^{31,36,37} has recommended disarticulation of the innominate bone with retroperitoneal dissection of iliac and obturator nodes. It is doubtful whether such extensive procedures, particularly disarticulation of the innominate bone, are indicated in such a malignant tumor as a melanocarcinoma. If it is necessary to do retroperitoneal dissection of iliac and obturator nodes to remove all palpable involved nodes, it is almost certain that other more distant areas of metastatic disease are present. For the same reasons, it is also questionable whether interseapulothoracic amputation should be done.

It is regrettable that a fair proportion of malignant melanomas of the skin arise in areas where lymph node drainage is unpredictable and where multiple areas of

TABLE 3
LOCATION OF EXCISED LYMPH NODES AND STATUS OF PATIENTS

LOCATION OF EXCISED NODES		INVOLVEMENT OF NODES	
		Clinical	Microscopic
Inguinal	17	7	9
Axillary	8	4	5
Cervical	15	6	6
Total	40	17	20

dissemination are possible. For instance, if a malignant melanoma arises in the midabdomen, the tumor can spread to the liver, axillas, or to the inguinal regions. The same applies for tumors located on the posterior midchest where supraclavicular, axillary, or even inguinal zones of dissemination are possible. For example, after dissecting both axillas for a tumor of the midposterior portion of the chest, unsuspected bilateral metastases were found in the axillas, and a few months later the patient developed supraclavicular lymph node metastasis.

It should be emphasized that if a node is palpable in a case of malignant melanoma, the chances are extremely high that it represents metastatic disease. It is not unusual to find metastases which are only 4 or 5 mm. in size. The large ulcerating type of malignant melanoma almost invariably presents metastatic disease. In 15 of our cases the nodes were considered clinically to be implicated, and in every instance the diagnosis was correct. The reason for this phenomenon is based on the method by which melanocarcinoma replaces nodes and, in particular, by the usual failure of melanocarcinoma to stimulate the production of connective tissue. Metastasis to a node may result only in very slight enlargement of the node, due almost invariably to tumor alone. The absence of palpable nodes does not militate against their involvement by disease. In two instances,

inguinal nodes thought by us to be negative were positive, but this incidence is usually much higher in other reported series. In 10 axillary dissections reported by Pack,³⁴ in which there were no palpable nodes, 5 were shown to contain metastatic melanoma. Pack³² also found that in two-thirds of the cases in which dissections were done, nodes were not palpable and tumor was found at the time of dissection; this was not further elaborated. Palpation of the axilla as a diagnostic procedure is most inaccurate because of the large volume and depth of this area. Deep nodes in the inguinal area are also difficult to palpate. The nodes in the cervical area are the most accessible, and therefore examination of this area is the most accurate. Of 40 patients, 22 died after radical excision and radical regional node dissection. Nine of these developed local recurrence, and *every one* had regional lymph node involvement. Six of these were in the inguinal region, a zone in which radical excision of lymph nodes is difficult. In 20 of the radical dissections in which implicated nodes were found, 7 had only one node involved, and an additional 9 had five or less.

FACTORS INFLUENCING PROGNOSIS IN MELANOCARCINOMA

NO EFFECT ON PROGNOSIS

- Sex of patient
- Microscopic pattern of tumor
- Amount of reticulum in tumor
- Amount of pigment in tumor and/or metastasis

GOOD PROGNOSIS

- Malignant melanoma occurring in a prepubertal child
- Freckle type of malignant melanoma (superficial lesion)

FACTORS THAT TEND TO GIVE A BETTER PROGNOSIS

- Subungual type of malignant melanoma
- Location in the head and neck area
- No previous treatment
- Radical surgical excision with regional node dissection

FACTORS THAT TEND FOR A POOR PROGNOSIS

- Location on lower extremities, trunk, or abdomen
- Involved but not fixed regional nodes; the greater the number involved, the worse the prognosis
- Previous inadequate treatment
- Satellite skin nodules

HOPELESS PROGNOSIS

- Distant metastases
- Persistent melanuria
- Fixed involved regional nodes

PROGNOSIS

The over-all prognosis of melanocarcinoma of the skin is extremely poor because a high percentage of the patients when first seen already have far-advanced disease (Table 4). Previous inadequate therapy (electrocauterization, carbon dioxide snow, or zinc chloride) had been administered in a high percentage of our patients; it definitely hastens death. This point was corroborated in 34 cases reported by Tod.⁴²

The sex of the patient has no bearing on prognosis, but the age is important. If a malignant melanoma appears in a child in the prepubertal age group and it is removed, the prognosis is excellent, even if the pathologic diagnosis is unequivocal. Pack³⁵ had 10 patients of prepubertal age all of whom survived. He states

TABLE 4

SUMMARY OF 75 CASES OF MALIGNANT MELANOMA INDICATING TREATMENT ADMINISTERED, PATHOLOGIC FINDINGS, NUMBER OF PATIENTS LIVING WITHOUT DISEASE AND NUMBER OF YEARS OF SURVIVAL

YEAR FIRST SEEN	NUMBER OF PATIENTS	NUMBER OF HOPELESS CASES	PALLIATIVE TREATMENT	EXCISION ALONE	EXCISION WITH REGIONAL NODE DISSECTION, NODES FOUND NEGATIVE	EXCISION WITH REGIONAL NODE DISSECTION, NODES FOUND POSITIVE	TOTAL LIVING WITHOUT DISEASE	SURVIVAL
								years
1939	8	● ● ●	● ● ●		○	○	2	8
1940	3	●	●			●	0	7
1941	19	● ● ● ● ● ● ●	●	●	● ● ● ○	● ● ● ● ○ ○	3	6
1942	13	● ●		● ● ●	● ● ○ ○ ○	● ● ●	3	5
1943	8	● ● ● ●			● ○	● ●	1	4
1944	10	●		● ○ ○	●	● ● ● ⊗ ○	3	3
1945	6	● ● ⊗			○	● ○	2	2
1946	8	● ●			○ ○ ○ ○	● ○	5	1
Total	75	23	5	7	18	22	20	

●, dead; ⊗ living with disease; ○, living without disease.

that the age group from puberty through 24 years has the worst prognosis. Recently MacDonald^{25a} reported melanocarcinoma in 6 patients (two boys and four girls) under the age of 15. Two had no metastases, three had regional node metastases and one had regional and distant metastases. The last subject was the only one who had died of the disease.

If melanuria due to malignant melanoma is present, the case is invariably far-advanced and the prognosis hopeless. Thirteen of our patients had melanuria consistently present and all of them are dead. It was hoped that the microscopic pattern of the tumor might be helpful in predicting prognosis, but unfortunately no correlation was found. The degree of pigmentation did not affect the prognosis. The amount of reticulum (Wilder's stain) apparently did not influence the outlook. It is possible that other factors, such as delay in diagnosis and previous inadequate treatment, so overshadowed these microscopic characteristics that they were not apparent. A large well-controlled series should be studied

from the viewpoint of pigmentation, dominant microscopic patterns, and amount of reticulum, in the hope that information similar to that discovered by Callender *et al.*⁶ for orbital melanoma might be uncovered. The freckle type of melanoma, because of its superficial character and its reluctance to metastasize, has the best prognosis of all morphologic types. The subungual melanoma is also a more favorable lesion to treat.

The prognosis of melanoma is not necessarily hopeless if immediate radical treatment is given. In 21 of our patients who have now been followed five years and who had radical excision with regional node dissection, 8 are living without disease (38 per cent). Three of the 8 had metastases to the regional lymph nodes at the time of operation. In two instances, the nodes were palpable, and in one instance in the inguinal area where a single node was involved, this node was not palpable. Twenty-two other patients were seen during the same period. Most had hopelessly far-advanced disease but several were given palliative therapy. All of these have expired. Our figures show an over-all 19 per cent five-year survival, but it should be stressed that a five-year follow-up does not imply cure in the skin melanocarcinoma, for 3 of our cases followed over five years died later of disseminated melanocarcinoma. Two others entered our hospital with involved regional nodes and distant metastases eight and fifteen years after excision of a malignant melanoma of the extremity. Those of our 75 patients who had a hopeless prognosis on admission lived an average of thirty and one-half months from the first symptom. In those patients treated, who subsequently died, the average duration of symptoms until death was approximately the same (twenty-eight months). DeCholnoky¹⁰ reported 2 patients, 1 dying eleven years and the other dying six and one-half years after primary therapy. Wilbur and Hartman¹⁶ also reported 7 skin melanocarcinomas which recurred between five and twelve years after original treatment. Prolonged survivals have been reported: Pack³⁵ reported 18 patients of his large group living ten to seventeen years; 7 out of the 18 had involved regional nodes.

Of 71 of our patients with adequate data and history, there were 35 to whom no treatment was given prior to admission here: 6 were hopelessly advanced on admission, but 29 were given palliative treatment; 13 of these 29 are still living. The average duration of symptoms was only nine and one-half months. In the remaining 36 of the 75 patients in whom treatment was given before admission, the average duration of symptoms in 30 cases was fifteen months, and in the other 6, was over forty-eight months. In this group of 36, 15 were hopeless, 21 were given definitive treatment, but only 5 are living. It can be seen that incorrect therapy delays curative treatment and thereby gives the disease a chance to spread. In 1936, Adair¹ reported 400 cases of malignant melanoma of which 155 received primary treatment. Fifty of these were far-advanced, leaving only 105 in which there was some opportunity for cure. Seventy of these 105 patients were followed for five years, and 23 (33 per cent) survived five years. There were also 55 operative cases for recurrences, out of which 15 (27 per cent) survived five years. In the entire group of 267 which had been followed five years, 38 were living (14.5 per cent).

The prognosis of malignant melanoma of the lower extremity is usually considered poor because of the difficulty in adequately removing the draining lymph nodes. This is doubtless true, but it is also true that the average duration of symptoms before treatment was eighteen months in our group, in contrast to ten and one-half months for melanomas of the head and neck.¹³ Furthermore, improper treatment which causes inevitable delay undoubtedly greatly contributes to the poor prognosis. If implicated nodes (cervical, axillary, or inguinal) are fixed, the prognosis is usually hopeless; also, the greater the number of involved nodes the worse the prognosis.

SUMMARY

A series of 75 cases of malignant melanoma of the skin has been studied. The difficulties of making a clinical and pathologic diagnosis of this highly malignant neoplasm have been discussed. Certain pathologic criteria valuable for diagnosis have been outlined, and the factors influencing prognosis have been detailed. In spite of the ominous character of this tumor, 8 of 21 patients (38 per cent) treated by radical excision and radical dissection survived five years. Three of the 8 had implicated regional lymph nodes. If 22 other hopelessly far-advanced cases which appeared during the same period are included, the percentage of survival for five years is 19 per cent. The treatment of this tumor must be radical surgical excision followed by radical dissection of regional lymph node areas when predictable. All forms of compromise therapy including irradiation are contraindicated.

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A RAPID METHOD FOR THE PREPARATION OF SEROLOGICALLY ACTIVE PHOSPHOLIPIN AND PURIFIED LECITHIN*

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Before the use of laboratory tests based on immunologic factors, the reports of studies of the phospholipids appeared in the publications of the biochemists. Erlandsen² in 1907 presented a comprehensive review of the literature and at the same time reported an intensive research on the phospholipids of beef heart muscle. It is interesting to note that the method used at the present time for the isolation and purification of lecithin is almost as described in that early paper.

The role of phospholipids as antigens in the serologic tests for syphilis was reviewed and summarized by Eagle¹ in 1937. Up to that time no worker had claimed success in isolating a single chemical compound possessing the necessary antigenic property.

Within the last decade Pangborn⁵ has prepared a phospholipid from beef heart muscle which, in combination with lecithin and cholesterol, has been successfully employed in serologic tests for syphilis. This so-called "cardiolipin" is apparently much nearer a chemical entity than the former lipoidal extracts used as antigens, but the intricate and time-consuming methods described for its extraction and purification have not made it practical to produce the substance in sufficient quantities for wide-spread study and subsequent adoption in routine serologic procedures.

The methods which follow have given satisfactory yields of lecithin and serologically active phospholipin, have not presented any problem in reproducibility of products and can be carried out in a period of approximately seven days.

EXTRACTION OF PHOSPHOLIPIN AND LECITHIN

Five beef hearts, weighing 4 to 5 pounds each, are used for the preparation of phospholipin and lecithin. The organs are removed immediately after the animals have been slaughtered. They are opened, drained rapidly and frozen with crushed carbon dioxide. (No more than thirty minutes should elapse between the death of the animals and the freezing of the tissue.) The hearts are kept in the presence of solid carbon dioxide, while the fat, connective tissue, blood and blood vessels are removed as completely as possible. The remaining heart tissue is cut into pieces while cooled with solid carbon dioxide. The cooled tissue is finely ground by repeated passages (8 times) through a meat grinder (one-eighth inch openings).

The wet, minced beef heart tissue, about 4 kilograms, is mixed with 3 liters of

* Received for publication, April 9, 1948.

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acetone per kilogram of tissue in a six-gallon crock. The mixture is stirred continually with an electric stirrer (1500 r.p.m. or more) for about twenty hours, then filtered by suction. The tissue is washed once on the filter paper with acetone; this extraction with acetone is repeated once. The acetone-extracted tissue, after being well drained, is then transferred to a special Eppenbach Q V 6-2 Colloidal Mill containing 3.5 liters of absolute methyl alcohol per kilogram of original wet tissue. Larger quantities of tissue cannot be satisfactorily extracted in a mill of this size. The mixture is ground and extracted for about four hours and then filtered by suction. The colloidal mill and the tissue on filter paper are washed several times with absolute methyl alcohol; then, the washed tissue is discarded.

To the clear, freshly filtered methyl alcohol extract is added a 20 per cent aqueous solution of barium chloride until no further precipitate is formed. The mixtures are allowed to stand overnight in the refrigerator. The supernatant extract is separated from the insoluble barium phospholipin by decantation and centrifugation and retained for the preparation of lecithin as described below.

PURIFICATION OF PHOSPHOLIPIN

The crude barium phospholipin precipitate is collected in 250 cc. centrifuge cups (40 cc. per cup) and washed once with 100–150 cc. of absolute methyl alcohol. After centrifuging, 80 cc. of ether, 20 cc. of absolute ethyl alcohol and 100 cc. of half-saturated sodium chloride solution are added and mixed thoroughly with a glass or porcelain spatula to dissolve the precipitate. The material is then transferred to a separatory funnel; the centrifuge cups are rinsed twice with a mixture of 40 cc. portions of ether, 10 cc. of ethyl alcohol and 50 cc. of half-saturated sodium chloride solution, and the washings are added to the material in the funnel. The two layers are separated without mixing, and the aqueous layer is discarded. The ethereal solution is treated with 20 cc. of ethyl alcohol for each 80 cc. of ether, followed by the addition of half-saturated sodium chloride solution equal in volume to that of ether-alcohol mixture. The solution is shaken vigorously for about five minutes. If an emulsion is formed, it is broken by the addition of small portions of ethyl alcohol and ether. The layers are separated, and the aqueous layer is discarded. The treatment with ethyl alcohol and half-saturated sodium chloride solution is repeated three times. The ethereal solution is then washed once more with half-saturated sodium chloride solution. The ethereal solution of sodium phospholipin is dehydrated overnight on about 100 grams of anhydrous sodium sulfate (Na_2SO_4), then filtered. The filtrate is concentrated by vacuum distillation to about 200 cc. or just to the point where the solid starts to separate from the ether. (In this and subsequent vacuum distillations the air is displaced by carbon dioxide, and the temperature of the water bath is kept at 37 C.) The cloudy solution is poured into about 1200 cc. of methyl alcohol with rapid mixing. The flocculent precipitate is separated by filtration, washed twice with 100 cc. of methyl alcohol and discarded. The solution is concentrated in vacuum to remove the ether.

To the methyl alcohol solution of partially purified sodium phospholipin is

added 20 per cent aqueous barium chloride solution until no further precipitate is formed. The end point is readily detected by chilling the mixture in ice. After standing overnight in the refrigerator, the precipitate is collected by centrifugation and washed once with methyl alcohol and once with acetone. To the precipitate is added 80 cc. of ether (anhydrous); then the latter is mixed thoroughly so that all the precipitate is transformed into a stiff gel. The gel is precipitated by the gradual addition of 80 cc. of acetone, and the ether-acetone purification is repeated until the supernatant is colorless. Four to six ether-acetone purifications are sufficient.

The barium phospholipin salt, purified by ether-acetone precipitation, is mixed with 100 cc. of ether followed by 20 cc. ethyl alcohol and 100 cc. of half-saturated sodium chloride solution; the whole is thoroughly mixed to dissolve the barium phospholipin precipitate and then transferred into a separatory funnel. The centrifuge cup is washed twice with a mixture of 100 cc. portions of ether, 20 cc. of ethyl alcohol and 100 cc. of half-saturated NaCl solution, and the washings are added to the material in the funnel. The mixture is shaken vigorously for about five minutes; the layers are separated, and the aqueous layer is discarded.

The treatment with sodium chloride solution is repeated three times, each time with the addition of alcohol in proportion of 20 cc. for each 100 cc. of ether remaining in the funnel and 300 cc. of half-saturated sodium chloride solution. The ethereal solution is then washed once more with the sodium chloride solution. The colorless ethereal solution of the sodium phospholipin is dried overnight on about 30 grams of anhydrous sodium sulfate salt, then filtered and concentrated by vacuum distillation to about 100 cc. With rapid mixing it is then transferred by means of pipet to 1200 cc. of absolute ethyl alcohol. (The flask is washed with small portions of ether which are added to the alcohol.) The slightly cloudy solution is concentrated in vacuum to about one liter. The small amount of flocculent precipitate which separates during distillation is removed by filtration.

If the alcoholic solution of sodium phospholipin is not entirely free from color, add an equal portion of absolute methyl alcohol, precipitate the phospholipin with 20 per cent barium chloride solution and repeat purification with ether-acetone precipitation described above.

The concentration of sodium phospholipin in alcoholic solution is calculated from the phosphorous content of this solution. The phosphorous conversion factor is 4.18 per cent. The sodium salt of phospholipin is soluble in absolute alcohol to 3.0 mg. per cc. at 1-3 C. and to 10 mg. per cc. at room temperature. It contains no nitrogen. The iodine number is 128-130.

PREPARATION OF LECITHIN

After removal of the crude barium phospholipin precipitate, 50 per cent aqueous solution of cadmium chloride is added to and mixed with the clear methyl alcohol extract until no further precipitate is formed. This mixture is allowed to stand in the cold overnight, and the precipitate is then collected and washed twice with absolute methyl alcohol and once with absolute ethyl alcohol.

PURIFICATION OF LECITHIN

To the 40 cc. of cadmium lecithin precipitate in centrifuge cup is added a mixture of equal parts of petroleum ether and 80 per cent ethyl alcohol. The solution is mixed thoroughly with a glass or porcelain spatula to dissolve the precipitate and transferred to a separatory funnel. The centrifuge cups are rinsed with a mixture of petroleum ether and 80 per cent ethyl alcohol. The total volume of petroleum ether and 80 per cent ethyl alcohol is about 500 cc. After a preliminary shaking by hand to release the air, the funnel is placed in a variable speed electric shaker for forty-five minutes and shaken with a motion simulating hand shaking. The alcoholic extract is separated from the petroleum ether so that no petroleum ether is added to the alcoholic extract. The above process is repeated four times using 500 cc. portions of 80 per cent ethyl alcohol and adding petroleum ether to keep the volume fairly constant.

The alcoholic extracts are combined and concentrated in vacuum to about 500 cc. (This process may be started during the extraction period.) A heavy precipitate of the lecithin cadmium salt separates as the petroleum ether is removed. The concentrated solution is allowed to stand in the cold overnight or longer. The precipitate is collected by centrifugation and washed once with absolute ethyl alcohol.

The purified cadmium lecithin salt is distributed in volume of approximately 25 cc. into 250 cc. centrifuge cups where it is washed once with 100-150 cc. of ethyl alcohol per cup. After centrifuging, 100 cc. of ether, 20 cc. of ethyl alcohol and 100 cc. of half-saturated sodium chloride solution are added, and the mixture is thoroughly stirred with a glass or porcelain spatula to dissolve the precipitate. It is then transferred to a separatory funnel, the centrifuge cups are rinsed twice with a mixture of 40 cc. of ether, 10 cc. of ethyl alcohol and 50 cc. of half-saturated sodium chloride solution, and the washings are added to the material in the funnel. The two layers are separated without mixing, and the aqueous layer is discarded. The ethereal solution is treated with 20 cc. of ethyl alcohol for each 80 cc. of ether, then with half-saturated sodium chloride solution equal in volume to that of the ether-alcohol mixture.

The solution is shaken vigorously for five minutes. If an emulsion is formed, it is broken by the addition of small portions of ethyl alcohol and ether. The layers are separated, and the aqueous layer is discarded. The treatment with ethyl alcohol and half-saturated sodium chloride solution is repeated three times. The ethereal solution of sodium lecithin is dried overnight on about 100 grams of anhydrous sodium sulfate, filtered and concentrated by vacuum distillation to approximately 100 cc. or just to the point where the solid starts to separate from the ether; then, about one liter of absolute ethyl alcohol is added, and the mixture is distilled in vacuum to remove the ether. The alcoholic solution of lecithin containing a small trace of color is filtered and purified once more. The entire process of purification (beginning with the cadmium chloride precipitation) is repeated to obtain a colorless alcoholic solution of sodium lecithin.

The concentration of sodium lecithin in alcoholic solution is calculated from

the phosphorous content of the solution. The phosphorous conversion factor is 3.95 per cent.

The sodium salt of lecithin is soluble in absolute ethyl alcohol up to 20 mg. per cc. at 1-3 C. and up to 100 mg. per cc. at room temperature. Nitrogen to phosphorous ratio is 1:1. The iodine number is 78-80.

REAGENTS

Barium chloride, 20 per cent, dissolved in water and filtered
Cadmium chloride, 50 per cent, dissolved in water and filtered
Sodium chloride, saturated solution. Half-saturated sodium chloride solution prepared from preceding solution and filtered
Sodium sulfate, anhydrous, C.P.
Acetone, C.P.
Methyl alcohol, anhydrous, C.P.
Ethyl alcohol, absolute, U.S.P.
Ethyl alcohol, 95 per cent, U.S.P.
Ethyl alcohol, 80 per cent
Ether, anhydrous, C.P.
Ether, petroleum, B.P. 30-65 C.

DISCUSSION

Early workers, as cited by Erlandsen, recognized the necessity for preventing enzymic activity and oxidation or other chemical changes resulting from exposure to air by keeping the tissues in solvents as much as possible or by operating at reduced temperatures. Solid carbon dioxide was chosen as the preservative agent since it prevents or greatly retards all enzymic or bacterial activity and chemical processes. Throughout the preparation these deleterious influences were minimized by exposing the material to atmospheric conditions as little as possible.

Care should be taken to carry out complete acetone extractions because residual acetone-soluble substances interfere with the precipitation of the phospholipin by barium chloride. This becomes evident from the formation of a sticky precipitate instead of the easily dispersed flocculent precipitate of the barium phospholipin salt.

As a means of preventing the undesirable changes in tissues, the Eppenbach Q V 6-2 colloidal mill was used. As originally designed, the apparatus stalled from clogging of the grinding mechanism by connective tissue. This resulted in overheating the material and slowing down the process, thereby causing significant losses of the sought-for phospholipin. There is now available a similar model of the colloidal mill* which incorporates a rotor and stator designed for micro-shearing action with a special extended shaft of fluted contour for the purpose of agitating suspended material in order to eliminate the necessity of using an auxiliary stirrer. The later model carries other mechanical improvements to minimize the hazards inherent in such equipment.

* This mill is manufactured and sold by Eppenbach, Inc., 45-10 Vernon Boulevard, Long Island City, New York.

Experience showed that the use of large volumes of ether in the ether-acetone purification process results in a pure product. An increase in the number of times that the ether-acetone purification was repeated with small volumes of solvents did not result in a product of like purity, probably because the solubility of the undesirable adventitious substances was decreased in the presence of relatively large amounts of the barium salt of serologically active phospholipin.

The formation of a precipitate during the solution of the sodium salt in absolute ethyl alcohol is due to one of two causes. If present in traces only, it is probably attributable to residual unconverted barium salt; large amounts of precipitate indicate that an insufficient amount of alcohol has been used. In this case the sodium phospholipin may be recovered by dissolving the precipitate in ether and adding it to a proportional volume of absolute alcohol.

For the chemical assay of the phospholipin and purified lecithin, any reliable quantitative analytic method for phosphorous, nitrogen and iodine number determination is applicable. The factors 4.18 and 3.95 for converting phosphorous content to phospholipin and lecithin are those of Pangborn.

The yields of serologically active phospholipin vary from 1 to 1.5 grams per kilogram of wet tissue; for lecithin, 4 to 5 grams per kilogram of wet tissue.

The phospholipin prepared by this method differs from the cardiolipin of Pangborn in that it is less soluble in absolute ether and absolute alcohol, and it is not precipitable as the cadmium salt. Weil and Ritzenthaler⁶ also found that the active antigen factor is not precipitable by cadmium chloride.

Assay of the six lots each of the phospholipin and purified lecithin prepared as described above in 1945 and 1946 by the author at the Venereal Disease Research Laboratory, U. S. Marine Hospital (USPHS), Staten Island, New York, has shown satisfactory and reproducible serologic reactivity in the complement-fixation procedures employed at that time in the same laboratory. Harris and his associates have reported the satisfactory use of these lipid antigens in microflocculation tests.^{3, 4}

The sodium salts of the phospholipin and purified lecithin in absolute ethyl alcohol solution preserved in brown bottles have remained stable for more than two years.

Phospholipin and purified lecithin, which from preliminary tests appear to be chemically and serologically identical with those obtained from beef heart, have been isolated from other animal tissue (liver, kidney, brain) and from some plants.

The term cardiolipin, therefore, appears to be too restrictive; at the present time, the substance prepared by this method is best referred to by the general name of phospholipin.

SUMMARY

A rapid method for the preparation of serologically active phospholipin and purified lecithin is described. The method gives satisfactory yields of purified lecithin and serologically active phospholipin. The lipid serologic antigens

PHOSPHOLIPIN AND LECITHIN

prepared by this method are chemically pure and readily reproducible; the process can be carried out in a period of approximately seven days.

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PARASITOLOGIC STUDIES OF WORLD WAR II VETERANS, WITH SPECIAL REFERENCE TO SCHISTOSOMIASIS JAPONICA*

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Inasmuch as a number of patients in this facility had served in World War II in regions highly endemic for schistosomiasis japonica, it was decided to undertake a parasitologic study in order to determine if asymptomatic or subclinical schistosomiasis could be detected among them.

METHODS

A series of 130 patients, who had served in or near areas known to be endemic for schistosomiasis^{1, 4, 5} and who had definite histories of exposure to fresh inland water in the endemic areas, was chosen for this study. In addition to the direct smear of the stool, several concentration methods were employed in this study. Repeated examinations were also made on each patient, not only because of the scarcity of ova during the chronic stage of the disease, but also because of the irregular appearance of the ova in the stools. Barody and Most² have shown in a series of daily examinations from 50 infected patients that from one to twenty-two days may elapse between two positive specimens. The following technics were used by us:

1. *Direct smear.* Bits of mucus, especially bloody mucus on the surface of the specimen, if present, were examined directly.

2. *Acid-ether concentration technic.* A portion of stool, weighing 5 to 10 grams, was comminuted in 25 to 50 cc. of 40 per cent hydrochloric acid containing a wetting agent and the material filtered through wet gauze. An equal quantity of ether was added, and the mixture stoppered and thoroughly shaken. It was then centrifuged for one minute at 1500 r.p.m. The material at the acid-ether junction was loosened and the acid and ether layers poured off rapidly. The sediment was then examined for schistosome ova.

3. *Sedimentation.* The remaining part of the specimen was comminuted in about 25 parts of tap water, containing 0.5 per cent glycerine, and filtered through wet gauze into a conical vessel and allowed to sediment from thirty to forty-five minutes. The supernatant fluid was poured off and the sediment resuspended. The process was repeated several times until the supernatant fluid was clear. The sediment was then examined for schistosome ova. The water Centrifugal Sedimentation Method, as described by Barody and Most,² was also used on some of the specimens.

4. *Egg-hatching technic.* Part of the sediment from the above procedure was poured into an Erlenmeyer flask which was then filled with chlorine-free water.

* Published with the permission of the Chief Medical Director, Department of Medicine and Surgery, Veterans Administration, who assumes no responsibility for the opinions expressed or the conclusions drawn by the authors. Received for publication, March 26, 1948.

It was allowed to stand overnight at room temperature and the surface examined for miracidia with a hand lens.

5. *Screening technic.* Since schistosome ova usually appear in the feces more infrequently and irregularly with the passage of time, it is desirable to achieve the maximum concentration possible. During the latter part of these studies, a technic was devised in which concentration of the ova was effected by a combination of screening and sedimentation. Fine mesh wire cloth is now available having remarkably uniform and exact sizes of sieve openings. Schistosome ova range in length from 74 to 106 or more microns and in width from 55 to 80 microns. The total specimen was thoroughly comminuted in tap water and filtered through 100-mesh wire cloth having openings of approximately 150 microns which removed particles larger than the size of schistosome ova. The "filtrate" containing the ova was allowed to sediment as in the sedimentation procedure described above. The final sediment was then filtered through 400-mesh wire cloth having sieve openings of approximately 37 microns which retained the ova and removed particles smaller than the schistosome ova. The material on the 400-mesh filter was washed with tap water several times to remove as many of the smaller particles as possible, resuspended in tap water, centrifuged and the sediment examined for ova. The concentrate is thus limited to particles having roughly the approximate size and specific gravity as schistosome ova. This method is advantageous in that the total specimen can be reduced to a relatively small volume. The wire cloth may be soldered onto the regular screening pans or cut in circles for use in the large size Seitz filter from which it may be easily removed for cleaning and re-use.

In addition to the above methods for recovery of schistosome ova, Faust's Zinc Sulfate Centrifugal Flotation Technic was used routinely for protozoa and other ova.

A comparison of methods was not attempted because of the small number of schistosome ova recovered from patients in the chronic stage of the disease.

RESULTS

Schistosoma japonicum ova were found in 5 of the 130 patients examined. Three infections were detected by the screening technic (Method No. 5). Hookworm ova were found in 44 patients, *Trichuris trichiura* ova in 34 patients, *Ascaris lumbricoides* ova in three patients, *Hymenolepis nana* ova in one patient, *Trichostrongylus* ova in three patients, *Strongyloides* larvae in one patient and *Endamoeba histolytica* in 18 patients.

COMMENTS

Inasmuch as an interval of at least two years had elapsed between the time of exposure to schistosomal infection and the time of the stool examination, infected individuals would have been in the chronic stage of the disease. That the recovery of the ova of *S. japonicum* constitutes the only method known of specific diagnosis both in the acute and the chronic stages of the disease has been stressed by Faust.³ Although repeated examinations using several concentration techniques

were made on each of the patients in this group, a relatively small number of schistosome ova were demonstrable. In each of the positive cases only a few ova were found, some of which were immature, and these occurred sporadically. Although the adult flukes continue to deposit ova for many years, the infrequent appearance of the ova in stool specimens is explained by the fact that as the disease progresses, an increasing number of ova remain in the intestinal wall or are carried to the liver and a decreasing number of ova are extruded into the intestine to be discharged in the feces. The high percentages of *S. japonicum* ova demonstrable in the native inhabitants of endemic regions may be due to the constant opportunity for reinfection from daily bathing or laundering in fresh water streams or from working in rice paddies. The high percentages of other parasites found in this group of patients studied is due to the known lack of sanitation in regions endemic for schistosomiasis.

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PROTEUS OX19 AGGLUTINATION IN PREGNANCY*

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In 1942 Gratch¹ reported that the serum of pregnant women gave a positive *Proteus OX19* reaction without regard to a history of or finding of rickettsial disease. One hundred per cent of his group of pregnant women had positive reactions, whereas the only false positive tests in the nonpregnant group occurred

TABLE 1

MAXIMUM TITER OF *Proteus OX19* AGGLUTINATION IN PREGNANT WOMEN IN RELATION TO MONTH OF PREGNANCY

MONTH OF PREGNANCY	NO. OF PATIENTS	TITER OF AGGLUTINATION						
		0	1:20	1:40	1:80	1:160	1:320	1:640
2	20	8	1	5	5	1		
3	23	7	5	4	6	1		
4	44	8	10	8	11	4	3	
5	25	6	7	5	3	2	1	1
6	26	7	5	7	5		1	1
7	27	7	3	9	4	4		
8	33	5	5	7	8	5	3	
9	9	6	1	2				
Totals.....	207	54	37	47	42	17	8	2
Percentages.....	100	26.1	18.0	22.7	20.3	8.2	3.8	0.9

TABLE 2

MAXIMUM TITER OF *Proteus OX19* AGGLUTINATION IN NONPREGNANT WOMEN

	TITER OF AGGLUTINATION						
	Totals	0	1:20	1:40	1:80	1:160	1:320
Number of patients.....	195	114	31	27	11	7	5
Percentage of patients.....	100	58.4	16.0	13.8	5.6	3.6	2.6

in the presence of malignant disease. He raised the question as to whether the reaction might not form the basis for a diagnostic test in pregnancy. White² in 1945 was able to confirm these findings partially, but we have noted no other reports in the American literature on this phenomenon.

Because of the potential value of this finding in the diagnosis of rickettsial infections or of pregnancy, this test was carried out in 402 women of whom 207 were pregnant and 195 were not.

* Received for publication, April 27, 1948.

Materials and methods. The 207 women were selected at random from those attending the prenatal clinic. The duration of their pregnancies, at the time of the tests, ranged from two to nine months. The nonpregnant women were outpatients of other clinics or inpatients of the hospital. Commercial *Proteus OX 19* antigen was employed. In all the tests here reported the slide technique was used, questionable clumping being checked under the microscope.

Results. Of the 207 tests on pregnant women, 73.9 per cent were positive to some extent. Table 1 summarizes the findings on this group and indicates that neither the percentage of positive reactions nor the height of the titer is statistically proportionate to the duration of the pregnancy.

Of the 195 nonpregnant patients some degree of agglutination was found in 81 or 41.5 per cent. The findings in this group are summarized in Table 2.

Comment. To a certain extent the number of false positive reactions found in the control group may be attributed to the fact that these women were selected from the clinics and wards of the hospital with diagnoses ranging from acute infections to malignant tumors. Nevertheless, a considerably higher percentage of the pregnant than of the nonpregnant patients gave positive reactions.

The data here presented would indicate that the *Proteus OX19* reaction as performed by us cannot serve as a reliable aid for the diagnosis of pregnancy. It does indicate, however, that the presence of pregnancy may render the test unreliable for the identification of rickettsial disease.

Conclusions. The serum of many pregnant women contains agglutinins against *Proteus OX19*. This finding, first observed by Gratch, is not sufficiently constant to serve as an aid in diagnosis of pregnancy. The frequent association of these agglutinins with pregnancy may interfere with the usefulness of the *Proteus OX19* reaction in the diagnosis of rickettsial disease during pregnancy.

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TROPICAL EOSINOPHILIA IN FILARIASIS

OCCURRENCE OF RADIATING PROCESSES ABOUT MICROFILARIAE*

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The occurrence of excessive leukocytosis and eosinophilia, with or without associated enlargement of peripheral lymph nodes and spleen, and pulmonary symptoms, particularly of asthmatic bronchitis, has been reported for many years, predominantly from the tropics. The term, "tropical eosinophilia", however, was first used by Weingarten¹⁸ who reported 81 cases of hypereosinophilia observed since 1934. In his series of cases no etiologic agent was found.

In some cases reported earlier by other authors, the available evidence pointed to parasites as the causative factor of the syndrome. De Langen¹⁰ observed several patients with asthmatic attacks, leukocytosis and marked eosinophilia who were infected with *Strongyloides stercoralis*. Meyers and Kouwenaar¹¹ found the microfilariae of *Filaria malayi* in the enlarged lymph nodes of patients with the same hematologic findings. The worms were lying in small abscesses containing eosinophilic leukocytes almost exclusively. In 1945 and 1946 van der Sar and Hartz^{15,16} reported two instances of tropical eosinophilia in which microfilariae, in all probability *Wuchereria bancrofti*, were found in enlarged axillary lymph nodes. In these instances the worms were also lying in eosinophilic abscesses or infiltrates. In a third patient, in whom the submental nodes and the nodes in the sulcus bicipitalis dexter were greatly enlarged, no microfilariae had been demonstrated at the time of their report; but they were found subsequently.

Meyers and Kouwenaar¹¹ and van der Sar and Hartz,^{15,16} reported that repeated examinations of the blood of their patients failed to reveal the presence of microfilariae.

Carter, Wedd and d'Abrera⁴ and Soysa and Jayawardena,¹⁷ all working in Ceylon, found larval mites, mostly Tyroglyphus or Tarsonemus, in the sputum of patients having tropical eosinophilia, but no enlarged lymph nodes. Their findings were confirmed by van der Sar¹⁴ at Curacao and by Wilson¹⁹ in East Africa. Therefore, it is very probable that the inhalation of dust, containing larval or sometimes adult mites, may also cause the syndrome of tropical eosinophilia.

The exact pathogenesis of tropical eosinophilia is not known. Microfilariae may be present in various regions without causing local tissue changes; when such changes are present, they may exist without tissue or marked blood eosinophilia.⁸ Furthermore, tropical eosinophilia develops in only a small fraction of the patients harboring microfilariae. It may, however, be safely assumed that hypersensitivity plays an important role. The diagnosis of tropical eosinophilia may be difficult since the microfilariae and larval mites can be found only by

* Received for publication, January 19, 1948.

careful and painstaking microscopic examination of excised lymph nodes or of sputum collected with special precautions. Especially in patients in whom the enlargement of the lymph nodes is the most conspicuous symptom, a clinical diagnosis of Hodgkin's disease may seem obvious. Sometimes, the atypical giant cells and the eosinophilic infiltrates may suggest the same diagnosis. In two instances (one instance not reported, in which the clinical diagnosis also was Hodgkin's disease) lymph nodes revealed numerous and greatly enlarged germinal centers, resembling giant follicular lymphoma,¹⁶ a finding which has been described in syphilis also.¹⁵ In still another case there was myeloid metaplasia in an axillary lymph node with eosinophilic myelocytes predominating.¹⁵

In view of the diagnostic difficulties just mentioned and since it has not yet become common knowledge that tropical eosinophilia may be caused by microfilariae, the publication of new cases seems justified. In addition, however, the case to be reported shows an interesting feature, that of radiate formation about the microfilariae.

REPORT OF CASE

A very obese woman, 30 years old, a native of Curacao, consulted a surgeon for a swelling between the left breast and axilla. Because of her obesity, palpation was difficult and an exact diagnosis could not be made. Therefore, surgical exploration was done; and a number of enlarged lymph nodes, partially covered by the pectoralis major muscle, were found and removed for microscopic examination. On gross examination the nodes were of different sizes, the largest measuring 2.5 x 2.5 x 1 cm. On section they were grayish red and showed numerous, very small, yellowish spots. A presumptive diagnosis of tropical eosinophilia was made and, accordingly, a blood count was done on the day after the operation. This showed 20,100 leukocytes per cu. mm. with 23 per cent eosinophils, 66 per cent neutrophils, 4 per cent lymphocytes and 7 per cent monocytes. No microfilariae were found in the blood.

The patient was given two injections of mapharsen. She was not seen again until five and one-half months after the operation at which time a blood count showed 11,300 leukocytes per cu. mm. with 4 per cent eosinophils. Roentgenograms of the lungs did not show anything abnormal. There were no enlarged lymph nodes and no subjective complaints.

Microscopic Examination

The tissue was fixed in Bouin's fluid. Paraffin sections were stained with hematoxylin-azophloxin, Masson's tetrachrome stain and Weigert's stain for fibrin.

The general structure of the nodes was preserved. In several places a marginal sinus could be recognized, and there were numerous distinct sinuses in the medulla. The cortex contained small follicles with here and there a small germinal center. The medullary cords were broad. Throughout the node, but especially in the medulla, the lymphocytes in the lymphoid tissue were almost completely replaced by plasma cells between which there were eosinophilic leukocytes in varying numbers.

The small yellow spots seen at gross examination proved to be infiltrates composed almost exclusively of eosinophilic leukocytes. They were located

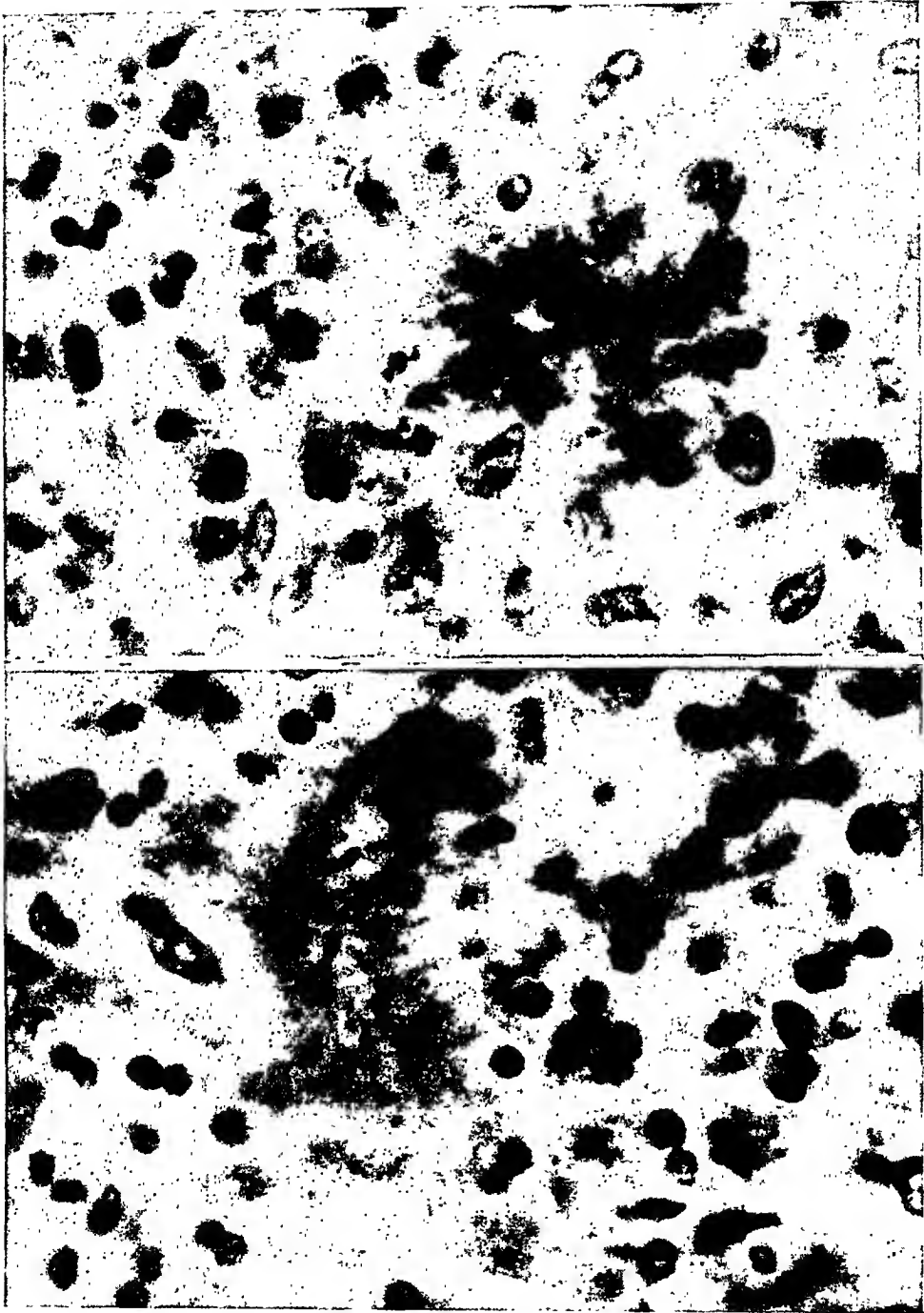


FIG. 1. Microfilaria in cross-section. Radiating substance is distinct. The worm is surrounded by histiocytes and eosinophilic leukocytes. $\times 1500$.

FIG. 2. Microfilaria with radiating substance in longitudinal section. $\times 1500$.

both in the cortex and in the medulla. The impression was gained that sometimes they bridged two or more medullary cords by completely filling up the intervening sinus or sinuses. The eosinophilic leukocytes in the centers of the infiltrates in places showed signs of degeneration, the nuclei being pyknotic and the granules staining pale red. They remained, however, always recognizable as eosinophilic leukocytes. In addition to this predominating cell type, the infiltrates contained histiocytes with pale vesicular nuclei. Sometimes, the infiltrates were sharply delimited; but, mostly, they merged imperceptibly into lymphoid tissue or medullary cords composed of plasma cells and containing numerous eosinophils. Eosinophilic leukocytes in great numbers were also present in the blood vessels and especially in the sinuses. True eosinophilic abscesses were not seen.

In many eosinophilic infiltrates typical microfilariae were found. They were somewhat shrunken, and in several there was a gradual disappearance of the nuclei. Many worms were surrounded by a thinner or thicker membrane of a homogeneous, oxyphilic substance. In one instance, closely adjacent to the microfilariae and enclosed in its membrane, there was a structure resembling a degenerated leukocyte. Processes of various shapes, thicknesses and lengths radiated from the membranes. Some were very thin and needlelike, others were curved and slightly swollen at the ends and still others resembled short clubs. Around other microfilariae they were irregular and sometimes appeared to have partly fused. In some worms sectioned longitudinally, the whole visible part was beset by radiating processes. The length of the processes rarely exceeded 8 to 15 microns. The Weigert fibrin stain did not stain the membranes or the processes. The latter were acidophilic and sometimes fairly refractile. In many places the radiating processes extended into a narrow zone composed of one or more rows of histiocytes with pale staining protoplasm between which there were a few eosinophilic leukocytes. The histiocytes were in turn surrounded by the leukocytes of the eosinophilic infiltrate. In other places the histiocytes were almost completely absent. In several places a giant cell closely bounded a microfilaria with its membrane and radiating substance or actually enclosed a microfilaria.

In a very few instances a degenerated microfilaria, recognizable by its form and the remaining nuclei, was surrounded by a thicker mass of oxyphilic substance, and the whole was engulfed by a giant cell.

Simultaneously with the disappearance of their nuclei, the worms became more eosinophilic, and in the final stages there remained only an oblong, red-staining structure. A few worms did not show the membranes with the radiating processes.

From the clinical point of view the present case is interesting in that it shows again that enlargement of lymph nodes in filariasis and *a fortiori* in tropical eosinophilia may present diagnostic difficulties, even in regions where the dif-



FIG. 3. Microfilaria in cross-section. Note thick membrane and short, club-like processes. $\times 1590$.

FIG. 4. Degenerated microfilaria, showing only one nucleus, enclosed in a giant cell, lying in a small granuloma. Also note short, club-like processes. $\times 1590$.

DISCUSSION

ferent complications of this helminthic infection are well known. Since in the present case the obesity of the patient rendered palpation difficult, and since she did not complain of asthmatic attacks or show symptoms of filariasis, a diagnosis of filariasis or of tropical eosinophilia was not considered. The presence of some kind of tumor of the chest wall or of a supernumerary breast was suspected. The gross appearance of the cut surface of the enlarged lymph nodes found at operation was typical of tropical eosinophilia caused by microfilariae, and this gross diagnosis was confirmed by the hematologic and histologic findings. The removal of the nodes and two injections of mapharsen apparently cured the patient, inasmuch as five and one-half months after the operation there were only 11,300 leukocytes per cu. mm. with 4 per cent eosinophils. However, it must not be forgotten that spontaneous regression of the symptoms of tropical eosinophilia may also occur.

Microscopically, the pathologic changes of the lymph nodes resembled those found in previous cases. There was extensive replacement of the lymphocytes of the medullary cords by plasma cells and eosinophilic leukocytes, and there were many eosinophilic infiltrates. However, a few differences also existed. In our previous cases the microfilariae found in the eosinophilic abscesses and infiltrates were lying in the midst of the eosinophilic leukocytes; and the atypical giant cells, when present, were lying outside the eosinophilic infiltrates, at least in the lymph nodes. In the present instance the worms were often confined to small granulomas, with or without giant cells, which in turn were lying inside the eosinophilic infiltrates. Sometimes, the worms seemed to be engulfed by the giant cells. In contrast to our previous cases, we could observe the disintegration of the worms with gradual disappearance of the nuclei and increasing eosinophilia.

Most interesting, however, were the radiating processes on the microfilariae. They varied in length and shape, and like the processes on sulfur granules of actinomyces they appeared as clubs, needles or sometimes as short irregular extensions. They radiated from an acidophilic membrane which surrounded the worms. The radiate formations were fairly refractile, strongly acidophilic and did not stain with Weigert's method for fibrin.

Radiating processes on disintegrating nematodes have been observed by us on previous occasions. In a case of human strongyloidiasis we have described short bands of a fibrinoid substance which seemed to radiate from the cuticular surface of degenerating filariform larvae lying in the submucosa of the colon.⁶ In another case in which a necrotic worm, probably an adult filaria, was found in the wall of the gallbladder, we have described a red-staining substance, resembling the clubs of *Actinomyces*, which seemed to radiate from the cuticle of the worm.⁷ Recently, we observed this radiate formation on microfilariae in a case of filarial epididymo-funiculitis with necrosis of the worms and an extraordinarily severe local eosinophilia. The slides from these cases were re-examined, and the radiating substance was found to have the same appearance and the same staining properties found in the present case. We, therefore, believe that the adjective "fibrinoid", used in connection with the radiate formation on the larvae of *Strongyloides stercoralis*, should be dropped.

Our finding of radiate formation on nematodes may throw some light on the problems connected with the origin of the radiating substance on pathogenic fungi. Moore,¹² in an exhaustive review of this subject, points out that this is not a species-specific or genus-specific phenomenon and emphasizes the fact that little is known about its origin and nature, which can be easily inferred from the many theories which have been advanced in this regard. The fact that morphologically identical formations have also been observed on nematodes may narrow the field of investigation.

Thus, the theory that the rays and clubs are living protoplasm, eventually capable of multiplying, becomes improbable. On the other hand, our findings certainly support the hypothesis of Berger, Vallée and Vézina¹ that the matter forming the actinomyeotic clubs is of extraparasitic origin and not an integral part of the fungi or of the botryomyeotic granules, as in their case.

It is interesting to speculate on the relation between radiate formation and inflammation and hypersensitivity. Moore¹² mentions the possibility of the radiating substance being related to Menkin's leukotaxine. To Henrici,⁹ studying experimental aspergillosis in rabbits, it seemed a justifiable assumption that the actinomycetoid form of the fungus and the tuberculoid character of the lesions resulted somehow from an allergic state. Though hypersensitivity is probably not essential for tubercle formation,¹³ it may, of course, have some influence on radiate formation. In this respect our findings are certainly suggestive. In all cases of radiate formation on helminths, there was a marked local eosinophilia, which in the present case and especially in our case of filarial epididymo-funiculitis, reached an extreme degree; and it is well known that hyper eosinophilia of the blood or of the different tissues is often related to hypersensitivity.^{2, 3} On the other hand, in our experience, local eosinophilia of such intensity occurs in filariasis only when necrotic worms are present, although not in all cases. This makes it very probable that some substances, liberated when the worms disintegrate, cause an allergic reaction in previously sensitized patients. How far this allergic or hypersensitive reaction can influence or cause radiate formation on helminths is an interesting subject for experimental research.

Another possible origin of the radiating substance is through digestive action of the host on the cuticle of the nematodes.¹² The fact that the cuticle of the filaria is very resistant and often forms the last recognizable remains of the worm, makes such an origin seem improbable.

SUMMARY

On surgical exploration of a swelling between the left breast and left axilla of a 30 year old, obese woman, a number of enlarged lymph nodes were found. Gross histologic and hematologic examinations showed the typical findings of tropical eosinophilia with microfilariae, many of which were disintegrating. These disintegrating worms showed radiate formations similar to those seen on some pathogenic fungi. The finding of radiate formation of helminths on previous occasions is mentioned, and the significance of these findings is briefly discussed.

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HUMAN ACTINOBACILLARY AND STAPHYLOCOCCIC ACTINOPHYTOSIS*

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Bodies that are similar to the granules of actinomycosis may be encountered in other fungus diseases, such as coccidioidomycosis and aspergillosis,^{12, 21} and in some bacterial diseases. Microscopically, these "granules" appear as lobulated bodies that stain faintly with hematoxylin and are bordered by radiate formations or club-like protrusions which take the eosin or saffron stain.

The granulomatous lesions, frequently found in the scrotal stump of the horse after castration by garroting, contain peculiar bodies surrounded by minute club-like excrescences. Bollinger⁴ in 1870 expressed the opinion that they were mycotic elements, and Rivolta²⁶ in 1887 proposed the name botryomyces, which has been used ever since. The true staphylococcic nature of these botryomycotic lesions was finally demonstrated in 1914 by Magrou.^{17, 18}

In 1902 Lignières and Spitz¹⁵ described an epizootic disease of cattle, "lumpy jaw", in which actinomycotic-like granules surrounded by clubs were present. This disease is caused by a short gram-negative bacillus which is now known as *Actinobacillus lignieresii*. In man bacteria may also occasionally give rise to the formation of "actinophytic" bodies in tissues. A perusal of the literature reveals 12 such cases reported up to 1947 (Table 1). In most cases, *Staphylococcus* was the causative organism, and in two instances, *Escherichia coli* was associated with a *Staphylococcus*. These cases were generally presented as examples of human "botryomycosis". Langeron¹³ in 1946 first described an infection in man produced by *A. lignieresii*.

Berger *et al.*³ in 1936 proposed that, in bacterial infections characterized by granules, shells or clubs, the term bacterial (*e. g.*, staphylococcic) actinophytosis be substituted for the term botryomycosis. The term, bacterial actinophytosis, follows the usage adopted by Lignières and Spitz who called the granuloma produced by their actinobacillus, actinobacillar actinophytosis. This change in nomenclature is now generally accepted.^{5, 6}

Four new cases of bacterial actinophytosis are reported here.

CASE 1

Hôtel-Dieu, No. A, 651. A white man, 63 years of age, was admitted to the hospital October 31, 1945, for dysuria and pollakiuria, hematuria and pyuria. For one year these symptoms had gradually increased, and on the day of admission painful micturition occurred every two hours. On cystoscopy a stone approximately 1 cm. in diameter was removed, and a large polypous growth of the bladder was seen, a fragment of which was taken for biopsy. The growth was a benign papilloma and was totally removed three days later after cystostomy. In the following weeks cardiac failure developed, and the patient died on December 3.

* Received for publication, March 2, 1948.

The gross anatomic diagnosis was purulent cystitis, bilateral purulent ureteritis and pyelonephritis, multiple renal abscesses, bacterial endocarditis of aortic valve, calcific sclerosis of aortic valve, mitral sclerosis with mild stenosis, hypertrophy of left ventricle, fibrino-hemorrhagic pericarditis (with exudate of 350 cc.) and severe passive congestion of lungs and liver.

Histologic examination. In the kidneys most of the purulent parenchymal foci contained 2, 4 and even 6 irregular, lobulated and often elongated bodies. Some of these bodies had

TABLE 1
LIST OF REPORTED CASES OF BACTERIAL ACTINOPHYTOSIS

AUTHORS	YEAR	NO. OF CASES	LESION	FOREIGN BODY	ORGANISM RECOGNIZED HISTOLOGICALLY	ORGANISM RECOVERED ON CULTURE
Kaiser and Gryns ⁷	1907	1	Osteitis of foot	Sequestrum	Staphylococcus	<i>Staphylococcus aureus</i>
Opie ²³	1913	1	Abcess of liver and lung	—	Staphylococcus	—
Masson ¹⁹	1918	1	Open fracture of thigh	Sequestrum	Staphylococcus	—
Fumigalli ⁶	1927	2	Chronic osteomyelitis	Sequestrum	Staphylococcus	—
Berger <i>et al.</i> ³	1936	1	Granuloma of labium majus	—	Staphylococcus and <i>Esch. coli</i>	<i>Staph. aureus</i> and <i>Esch. coli</i>
Plaut ²⁴	1937	1	Abcess of abdominal wall	Broom-corn plant	Staphylococcus	—
Kimmelsteil and Oden ¹⁰	1939	2	Granuloma of omentum	Fish bone	Staphylococcus	<i>Staph. aureus</i> and <i>Esch. coli</i>
			Granuloma of abdominal wall	Fish bone	Staphylococcus	—
Fink ⁵	1941	1	Abcess of liver and lung	—	Staphylococcus	<i>Staph. aureus</i>
Langeron ¹³	1945	1	Granuloma of face	—	Actinobacillus	—
Moulonguet and Gassuc ²²	1946	1	Kidney	—	Staphylococcus	—

been broken by the microtome knife. Most were solid, but some had a hollow center containing a few pyknotic or degenerating polymorphonuclears. In the routine sections, the centers of these bodies were faintly stained by hematein, and their irregular peripheries were stained by erythrosin. The peripheral swellings varied greatly in size, some being short and oval, others elongated and rod-like. They were stained red by basic fuchsin and blue by Krajan's technic. On many, minute secondary formations were present, giving a fish-bone appearance. In the gram-stained sections no gram-positive mycelia or bacteria could be identified in the "granules". Their centers did contain, however, many loose

bundles of bacilloid elements which were distinctly stained red by Pappenheim's pyronin-methyl-green and Krajian's bacterial stain and blue by Giemsa's stain and methylene blue (Fig. 4.). These minute rods, undoubtedly bacterial, were generally too short and too broad to be *Escherichia coli*. Their morphology resembled that of an actinobacillus.

No similar gram-negative rods were present outside the "granules" found in the purulent foci of the kidneys. Multiple sections taken from other organs did not contain similar "granules", and no bacteria in any way resembling actinobacilli were seen in inflammatory foci elsewhere.

Bacteriologic examination. No purulent material from the kidneys was collected at autopsy for bacteriologic study. From the pericardial exudate, however, there was isolated

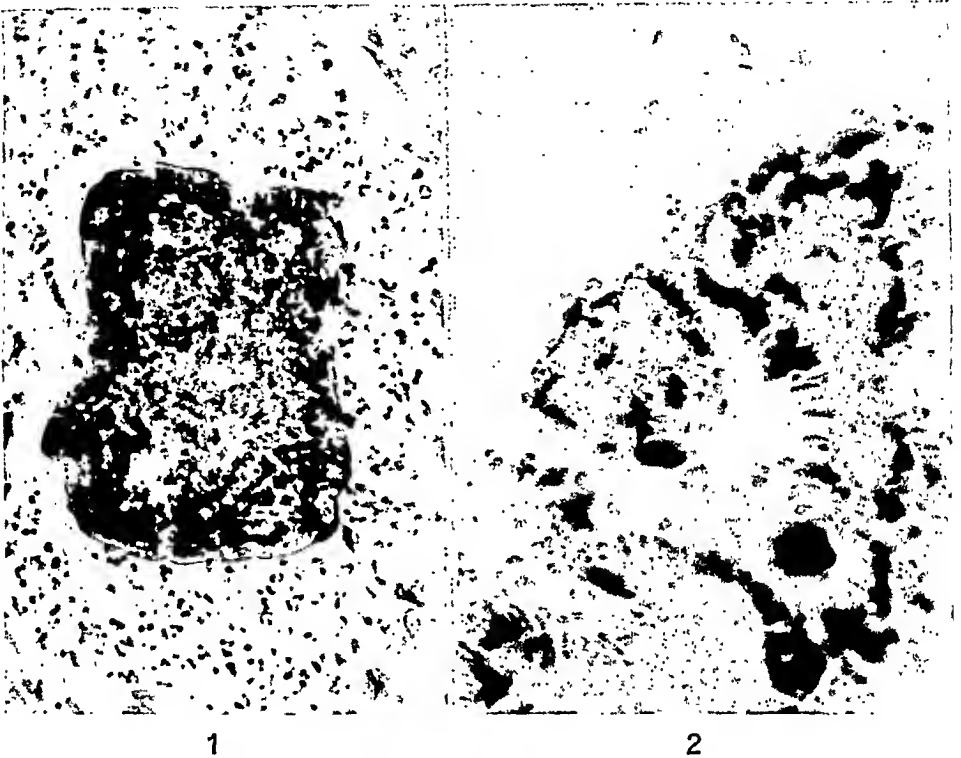


FIG. 1. Case 2. Staphylococcic actinophytosis. "Granule" limited by a cuticular shell. $\times 420$.

FIG. 2. Case 3. Staphylococcic actinophytosis resembling actinomycotic granule. $\times 420$.

a pure culture of a small gram-negative cocco-bacillus, identical in length and in diameter to the rods seen in the renal masses. This organism measured 1.0μ by 0.5μ . It grew best at 37°C ., but fairly well at 20°C . A facultative anaerobic, it formed on agar slant, small, smooth, grayish white and translucent convex colonies. In peptone broth a slight turbidity appeared with a deposit after a few days. Poor growth occurred in gelatin stab without liquefaction. This bacterium acidified but did not coagulate milk, did not form indole in tryptone broth, reduced nitrates, but showed no hemolytic activity on blood agar. Its biochemical activities included acidification without gas formation of dextrose, lactose, saccharose, levulose, maltose, mannite, mannose, galactose and salicin, and production of hydrogen sulfide on lead-acetate agar. The isolated organism was not pathogenic for the mouse, the rat, the guinea pig or the rabbit. The general bacteriologic characters of this

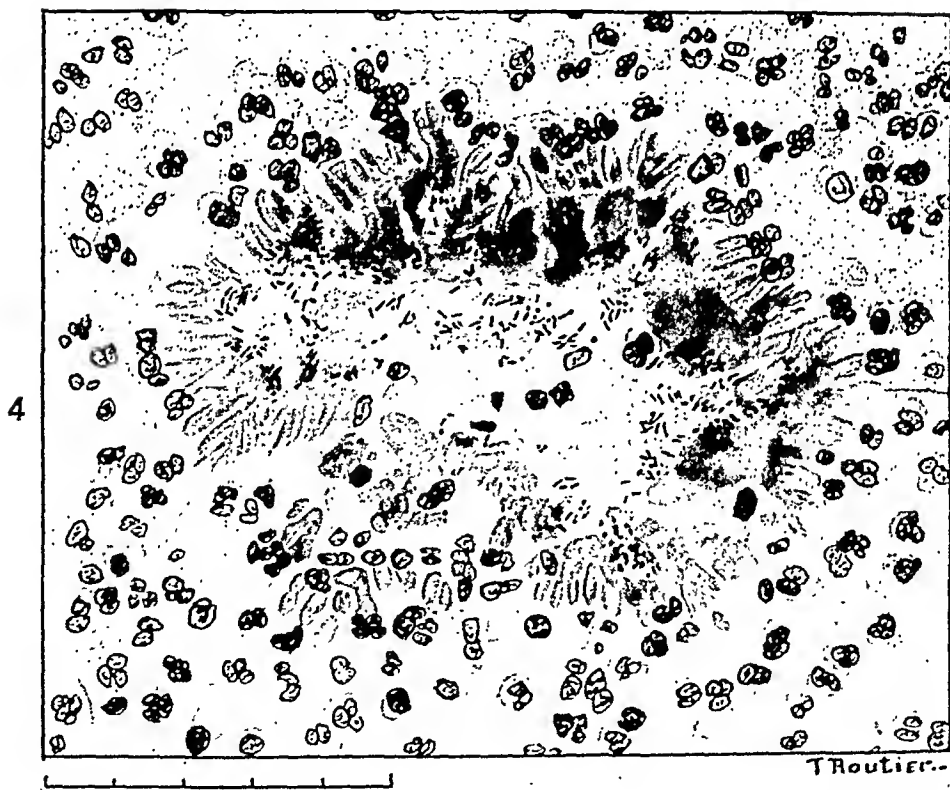
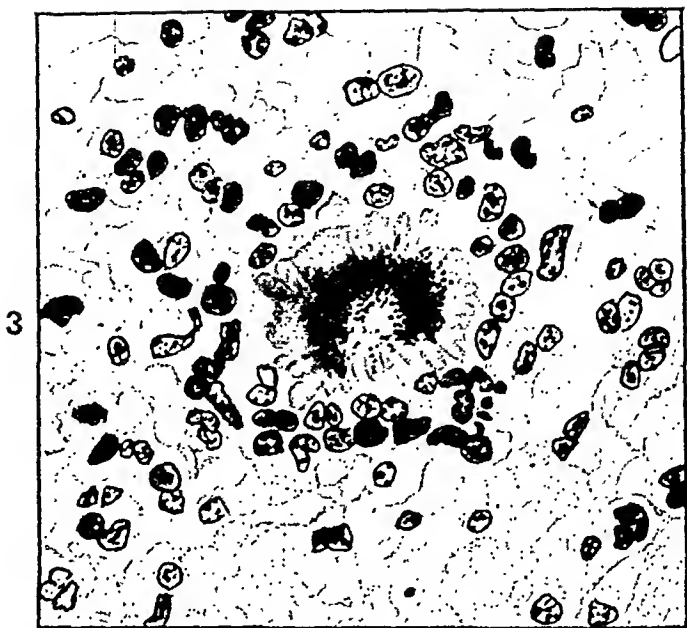


FIG. 3. Case 4. Staphylococcic actinophytosis. "Granules" surrounded by budding clubs. $\times 800$.

FIG. 4. Case 1. Actinobacillar actinophytosis. "Granules" with irregular peripheral protrusions, containing many small bacilloid elements. $\times 740$.

cocco-bacillus are comparable to those given by Lignières and Spitz for *Actinobacillus lignieresii*.*

* This identification was made by Dr. L. Gauvreau and Dr. H. Turcotte of the Department of Bacteriology of Laval University. Full bacteriologic data in this case will be published elsewhere.

CASE 2

Hôtel-Dieu, No. 18266. A 57 year old man was admitted to the hospital for intestinal obstruction caused by volvulus, and a colostomy was performed. This was closed a few weeks later at which time a small segment of colon attached to a fragment of skin was removed for biopsy.

Histologic examination. In one of the sections between the epidermis and the intestinal mucosa there was a large area of granulation tissue covered by necrotic tissue and pyogenic exudate. In the exudate there were two rounded bodies, approximately 80 μ and 100 μ in diameter, surrounded by a small focus of polymorphonuclears (Fig. 1).

In the routine stained sections these bodies resembled actinomycotic granules, but no peripheral clubs were present. Staining by Gram's method revealed no mycelia, but the centers of the granules were made up of dense clumps of minute round and regular particles, most of which were strongly gram-positive, with a few here and there taking the stain lightly or not at all. These particles, which had characteristics of cocci, were embedded in homogeneous substance that stained pink with May-Grünwald-Giemsa stain and red with carbol-fuchsin, but were not acid-fast. The "granules" were limited by a definite acellular cuticular membrane, which stained deep blue by the Dominici-Mann-Masson method. With the May-Grünwald-Giemsa stain, this membrane was also blue and could be easily identified by contrast with the pink color of the basic substance which contained the cocci. On the basis of size and arrangement the particles appeared to be staphylococci.

CASE 3

Hôtel-Dieu, No. 24806. A 23 year old man was admitted to the hospital for a mass in the anterior region of the right side of the neck. This mass, as large as a medium-sized orange, was hard but not painful, and had been observed by the patient to develop during the course of one month from the size of a pea. It was not adherent to the skin, moved slightly when the patient swallowed and appeared to have arisen in the right sternocleidomastoid muscle. The temperature and pulse rate were normal. A surgical incision revealed a large abscess surrounded by a thick fibrous capsule. A fragment was taken for pathologic examination but no material was collected for bacteriologic study.

Histologic examination. A section of the excised fragment consisted of a band of sclerotic skeletal muscular tissue. It was infiltrated by a small number of lymphocytes, plasmocytes and histiocytes and bordered on one side by granulomatous tissue which in turn was covered by polymorphonuclears.

In the granulomatous tissue there were three bodies or "granules" bordered by club-like swellings which resembled actinomycotic granules (Fig. 2). These bodies were irregularly lobulated. The peripheral clubs were quite uniform in size and pear-shaped and resembled actinomycotic clubs, being pink with erythrosin and deep red with magenta-picro-indigo-carmin stain and Krajan's bacterial stain. They seemed to arise from a thin circular band of homogeneous substance which showed the same staining properties as the clubs. In the center of the "granules", however, no gram-positive mycelia were present, but only a moderate number of coccus-like bodies. These small spherical bodies were found in small, dense clusters, did not take Gram's stain, but stained blue with May-Grünwald-Giemsa stain and magenta-picro-carmin stain, red with Pappenheim's pyronin-methyl-green stain and deep red with Krajan's stain. They were embedded in a light, amorphous matrix which stained yellow with magenta-picro-indigo-carmin and pink with Krajan's stain. The size and grouping of these cocci did not resemble segmented mycelia, but rather staphylococci. They were, however, totally gram-negative. The histologic appearance was that of actinophytic bodies with central degenerating or dead staphylococci.

After the sections had been studied, a curettage of the lesion was made through the surgical incision, and the material obtained was spread on various culture mediums. No significant pathogenic bacteria were isolated. *Bacillus subtilis* grew on a few mediums and *Staphylococcus albus* on some others, but most of the mediums remained sterile.

CASE 4

I.A.P. 38954. For a few weeks prior to admission to the Quebec Military Hospital a 25 year old army officer had complained of loss of appetite, digestive malaise and general fatigue. For two days prior to admission he suffered sharp and persistent abdominal pains in the right lower quadrant and in the para-umbilical region. The patient's appendix had been removed five years previously.

There was marked contracture of the abdominal wall, and a deep-seated, large, irregular and tender mass was palpated with difficulty to the right of, and beneath, the umbilicus. The temperature was 100 F., the pulse rate 96 and the leukocyte count was 16,600 per cu. mm. with 87 per cent polymorphonuclears.

At operation the omentum was found to contain a large hemorrhagic, necrotic and purulent mass, which was firmly attached to the transverse colon for a length of 5 cm. and covered most of the small intestine. While liberating this mass, many small purulent foci were opened; and a small opening, approximately 8 mm. in diameter, was found on a loop of the ileum. The surgical diagnosis was secondary epiploitis, in all probability due to an acute perforating diverticulitis. The resected epiploic mass measured 8 cm. in length.

Histologic examination. Many sections taken from the epiploic mass revealed a diffuse subacute inflammatory infiltrate with anastomosing strands of fibrous tissue and numerous necrotic, hemorrhagic or purulent areas. In most of the purulent foci there were rounded bodies or "granules", approximately 50 μ in diameter (Fig. 3.), whose centers stained slightly with hemalum and by Gram's method and consisted of dense colonies of small gram-positive cocci. The periphery of the granules was formed by acellular material with an irregular frayed or cog-wheel-like edge that gave the impression of numerous budding clubs. This material was stained red by erythrosin, by Krajian's bacterial stain,¹¹ and by carbol-fuchsin using a modified Ziehl-Neelsen technique in which the slides were passed through 10 per cent acetic acid and absolute alcohol. The granules were surrounded by a moderate number of pyknotic polymorphonuclears and a thin outer layer of fibrin.

No material was obtained for bacteriologic study, but throughout the tissue there were scattered small clumps of gram-positive cocci which had the morphologic characteristics of staphylococci.

COMMENT

The mechanism of actinophytic evolution in bacterial infections is not well understood. When "granules" and clubs in tissue were thought to be pathognomonic of actinomycotic lesions, these formations were considered as simple morphologic variations of the mycotic threads themselves. But since radiate formation has been reported on dead actinomycotic filaments,²⁰ on various bacteria including acid-fast bacilli¹⁶ and on inorganic particulate matter such as tellurium,¹⁴ this widespread phenomenon cannot be attributed solely to a change in morphology of the causative agent. An important participation of the host seems highly probable. A view, first sponsored by K. Meyer²⁰ in 1934 and since accepted by others,³ is that granules, shells and clubs are the result of a coagulation or precipitation of protein substances around a pathogenic agent, brought on by an interaction between the agent or its toxin and the surrounding proteins. This interpretation covers also the formation of granules in culture mediums.

In the first cases of human bacterial actinophytosis reported, the role of the foreign body was stressed.^{6, 19} The lesions were in osseous tissue, and the presence of a sequestrum was believed to have protected some cocci from phagocytosis and, therefore, permitted the formation of granules. Kimmelsteil and

Easley⁹ experimentally produced staphylococcic actinophytosis in rabbits by the insertion of fish-bone fragments in the intestinal wall. They concluded that the presence of this foreign body perpetuated the infection and was a preponderant factor in the development of the granules. In our four cases and in cases reported elsewhere,^{3, 5, 23} no foreign body was demonstrated.

Our Case 1 is, to our knowledge, the second instance of human actinobacillary actinophytosis to be recorded. Langeron in 1941¹² reported a similar infection in a 16 year old French girl. In a granulomatous lesion of the right side of the face and neck, a "granule" was found. Its periphery was formed by irregular rod-like swellings and its center by gram-negative bacilloid elements. According to this author, the histologic aspect was typical enough to permit a diagnosis of actinobacillosis, even if no material was obtained for bacteriologic study and no bacillus isolated. In two instances of actinobacillosis reported in the United States^{2, 27} and one in France²⁵ there were granulomatous lesions or bacteremias, but no "granules" were demonstrated. These instances cannot, therefore, be classified as actinobacillary actinophytosis.

SUMMARY

Four instances of bacterial actinophytosis were encountered in lesions of soft tissue. In one case *Actinobacillus lignieresii* was responsible and in the other three it was believed that staphylococci were the causative agents.

Colonies of bacteria occurring in tissue may be indistinguishable from actinomycotic granules in routine stained sections. The use of bacterial staining methods may establish the histologic diagnosis.

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LOWER NEPHRON NEPHROSIS ASSOCIATED WITH MASSIVE ADRENAL INFARCTION*

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The association of lower nephron nephrosis with hemorrhagic infarction of the adrenals is unusual and warrants attention.

The term, lower nephron nephrosis, has been given by Lucké⁴ to the distinctive renal lesions found in a variety of conditions, which have in common destruction of tissues or blood and which lead clinically to acute renal insufficiency. Hemoglobinuric nephrosis, crush syndrome, traumatic anuria, renal anoxia or tubulovascular syndrome of Maeraith^{5, 6} are other descriptive terms which have been applied.

The histopathology is that of degeneration in the distal segments of the nephron associated with heme casts in the collecting tubules. Mallory,⁷ as a result of observations on the development of hemoglobinuric nephrosis in traumatic shock of battle injuries, stresses that two factors of paramount importance are pigment excretion and recovery from a state of shock or a related physiologic abnormality. The lesions of this type of nephrosis have been noted following severe trauma to muscle, nontraumatic muscle ischemia, burns, heat stroke, uteroplacental damage, intravascular hemolysis due to incompatible blood transfusions, black-water fever, favism, intoxication due to sulfonamides, a variety of poisons, and "shock" from other causes.

REPORT OF CASE

Clinical Data

M. A., a 28 year old woman, Para II, was admitted to the hospital on October 2, 1947 because of vaginal bleeding. When the patient was two months pregnant, she developed abdominal pain which was followed immediately by vaginal bleeding. She was then kept in bed for one week, but the persistence of bleeding necessitated hospitalization. Bleeding had started spontaneously, and the use of abortifacients was emphatically denied. Immediately after admission to the hospital, the patient passed some tissue which was diagnosed on microscopic examination as endometrium showing "decidual reaction".

The patient previously had been in good health, having had only the usual childhood illnesses. Her confinements had been entirely normal. Previous urinalyses had been entirely negative, and prenatal examination in September 1947 had revealed a normal blood pressure.

On October 5, 1947, curettage of the uterus was attempted. After preoperative medication of morphine gr. $\frac{1}{4}$ and atropine gr. 1/150, the cervix was dilated under chloroform and ether anaesthesia, and the curet was inserted. Immediately, the patient commenced to bleed profusely and "went flat". The uterus was packed without completing the curettage, and the patient was returned to the ward. Following the attempted operation, the temperature, pulse rate and respiratory rate rose and remained elevated until death. No

* Received for publication, December 24, 1947.

urine was passed for eighteen hours, after which time, oliguria was noted. Despite the administration of glucose intravenously and alkaline salts orally, the urinary output dropped to "anuric levels" by the fifth post-operative day.

On October 8 the patient suffered a severe chill and again started to bleed vaginally. At this time the patient was markedly dyspneic and complained of numbness in her hands and feet. Examination of the blood revealed a hemoglobin level of 6.5 Gm. (Sahli), a red cell count of 2.2 million and a leukocyte count of 8700. The patient's blood was group A, type Rh-positive. Sulfathiazole was administered intravenously, 1 Gm. every four hours for one day, and 500 cc. of compatible blood was given. Despite a blood transfusion and administration of oxygen, the patient remained dyspneic.

Scanty granular casts and protein appeared in the acid urine. The nonprotein nitrogen of the blood rose to 78 mg. per 100 ml. on October 10, and the carbon dioxide combining power of the blood fell to 38 volumes per cent. Blood pressure was recorded on October 9 and 10 as 79/36 and 97/68, respectively. Bacteriologic studies were negative. The patient expired on October 11, 1947, six days after operation.

Macroscopic Postmortem Findings

Autopsy was performed two hours after death. The gross findings were as follows:

The body was that of a well developed young woman. Considerable mucus was present in the bronchi, and there was associated atelectasis of the lower lobes. The *lungs* each weighed 310 Gm. and, apart from the atelectasis, were grossly negative. The *heart* weighed 345 Gm. and showed minimal diffuse fibrosis of the myocardium and minimal atherosclerosis of the coronary arteries. A minimal degree of atheroma of the *aorta* was present. Eight cm. distal to the aortic ring at the site of the origin of the fibrosed ductus arteriosus, there was a narrowing, at which point the aortic lumen measured 0.6 cm. in diameter. There was no associated enlargement of other arterial channels in the thorax. The *spleen* was firm, congested and weighed 380 Gm., a degree of enlargement not uncommon in pregnancy. The *liver* was reddish brown in color with retention of liver markings and weighed 2100 Gm. The *uterus* was enlarged to the size of a three month pregnancy and still contained the greater portion of the fetus and practically all of the placental tissue. There was no retroplacental hemorrhage. The parametrial veins were free of thrombi. A dark red blood clot destroyed both *adrenals* and gave the appearance of delimited molds of blood. The combined weight of the adrenal casts was 40 Gm. No thrombi or emboli were demonstrated in the adrenal vessels (Fig. 1). The *kidneys* were swollen but of normal consistency. Each kidney weighed 275 Gm. and its capsule stripped easily revealing a pale, smooth surface. The cortex was moist, bulging, pale yellow and measured 1.2 cm. in thickness; in contrast the medulla was dusky with accentuated dark red striations (Fig. 2). The brain, pituitary, thyroid, pancreas, lymph nodes, gastrointestinal tract and urinary bladder were essentially negative.

Microscopic Findings

Lungs. Atelectasis was noted in one section, but the remainder of the lung parenchyma was negative. *Heart.* No myocardial changes were encountered except for a minimal amount of paravascular fibrous tissue. No inflammatory

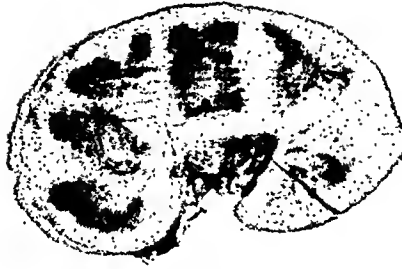


FIG. 1. Diffuse hemorrhage into adrenal. $\times \frac{1}{3}$.

FIG. 2. Kidney showing marked cortical swelling and pallor with congestion of medulla. $\times \frac{1}{3}$.

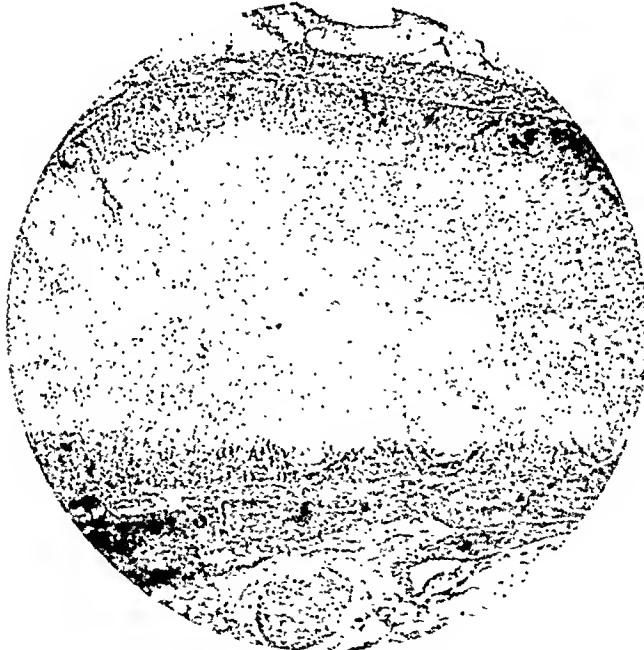


FIG. 3. Adrenal showing complete infarction, peri-adrenal and cortical hemorrhage and line of leukocytic reaction. $\times 13$.

foci were discovered. *Liver.* The liver cells showed minimal cloudy swelling. There was slight infiltration of lymphocytes and an occasional polynuclear and mononuclear cell in the portal spaces. No regions of necrosis were found.

Adrenals. The cortex and medulla showed complete infarction with wiping out of cytoplasmic detail and disappearance of nuclear material. Extravasation of red cells was dense in the zone of the erased cortex. This hemorrhagic zone was partially delimited by a scattering of polynuclears. The cortical capillaries were stuffed with hemolyzed red cells. Periadrenal congestion and hemorrhage were noted (Fig. 3). *Spleen.* Congestion of splenic sinusoids was found, malpighian follicles were moderately prominent, and a few polynuclears were scattered through the pulp.

Kidneys. The glomerular tufts were full and free from any intrinsic endothelial change. Some of the capsular spaces contained eosinophilic amorphous debris. The proximal convoluted tubules showed albuminous degeneration, and the lumens held eosinophilic flocculent amorphous material. The distal convoluted tubules and ascending limbs of Henle contained eosinophilic casts, occasionally ribboned or finely granular. Pale eosinophilic hyaline casts were present, mixed with orange-colored granular casts or isolated as intra-luminal units. Numerous pinkish orange casts were found in the collecting tubules. Engorgement of the vasa recta was present. There was an occasional inter-tubular communication with a contained cast, and the odd connection could be interpreted as a tubulovenous rupture. There were patchy formations of young granulation tissue with a scattering of lymphocytes, mononuclear cells and occasional extravasated erythrocytes (Figs. 4-7).

This picture is indistinguishable from lower nephron nephrosis described in other conditions. There was no evidence of antecedent renal disease. *Uterus and retained products of conception.* The decidual tissue and chorionic villi showed numerous infarcts. The point of attachment of the placental tissue to the uterine wall showed only a narrow zone of demarcating leukocytes. There was no granulocytic reaction of hemorrhage in the uterine wall. *Bone marrow.* Slight hyperplasia was found with cells of the normoblastic and granulocytic series sharing equally. Megakaryocytes were not unusual. Other organs were essentially negative on microscopic examination.

COMMENT

The genesis of the primary etiologic factor of this case is masked. A hemolytic reaction cannot be incriminated as the causative factor in the production of the nephrosis, since oliguria preceded the blood transfusion by three days. Sulfonamide as the noxa can be ruled out for the same reason. Intravascular hemolysis with a resultant lower nephron nephrosis has been reported following the use of quinine and soft soaps as abortifacients due to their lytic action on the red blood cells. The patient, however, strongly denied the use of abortifacients in any form. The uterus was not infected nor was there evidence of criminal interference. There was insufficient uteroplacental damage to support Young's⁹ thesis of retroplacental hemorrhage as an explanation for the renal insufficiency.

The lower nephron nephrosis in this case is a sequela of the operative shock and the infarction of the adrenals. It cannot be definitely stated which of these conditions is the primary etiologic agent. The cause of the massive infarction of



FIG. 4. Granular cast in renal tubule with marked congestion of vasa recta. $\times 260$.
 FIG. 5. Casts in renal tubules with intertubular venous communication. $\times 500$.
 FIG. 6. Fragmentation of cast with granulomatous reaction. $\times 260$.
 FIG. 7. Granulomatous focus in kidney with lymphocytes, mononuclears and extravasated red blood cells. $\times 260$.

the adrenals is obscure. The sudden fall in blood pressure at the time of operation may have been a factor. Blood cultures were consistently negative. The narrowing of the aorta was not associated with any of the antemortem functional sequelae or postmortem collateral circulation.

The renal cortical pallor and the congestion of the vasa recta can be interpreted as evidence to support the more recent emphasis that has been placed on renal anoxia. This disturbance in renal blood flow is considered to be the important mechanism in the production of the syndrome of lower nephron nephrosis.

Barclay *et al.*¹ in animal experiments have produced a diminution in renal cortical blood flow by arterial spasm due to overstimulation of vascular nerves and have demonstrated in a dramatic fashion a shunting of the renal circulation through an alternative route in the medulla leading to renal anoxia. The sequence briefly is that of shock, shunt and shut down.

In the light of these researches, W. W. Woods⁸ suggests that the sudden engorgement of the vasa recta of the medulla may lead to rupture of these veins into the adjacent tubules with the formation of tubulovenous communications, the latter of which may be largely responsible for the clinical and pathologic features of lower nephron nephrosis. The previous implications by Dunn³ and Bywaters² were that these communications were formed by a break-through from tubules to vein.

SUMMARY

1. Death from lower nephron nephrosis associated with cryptogenic hemorrhagic infarction of the adrenals is reported.

2. The cortical pallor, medullary congestion and tubulovenous ruptures of lower nephron nephrosis can be interpreted in the light of recent experimental work as evidence of renal anoxia.

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CLINICOPATHOLOGIC CONFERENCE*

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CLINICAL DATA

Presented by Dr. John Connell

"History. B. A. K., a $3\frac{1}{2}$ year old girl, entered the hospital in April 1946 for traction for a deformity of the right lower extremity, a residuum of acute poliomyelitis contracted in 1944. She had been born at full term and progressed rather slowly during the initial weeks on a variety of artificial formulas. At three months of age she was admitted to the hospital because of a feeding problem, having failed to gain weight. At that time her weight was 8 pounds 6 ounces. Physical examination, excepting for moderate malnutrition and excoriation of the buttocks, was essentially normal. She was next admitted to the hospital at the age of 9 months for treatment of acute bronchitis. From the time of this admission until death she had a persistent cough. In all she was admitted to the hospital on 8 occasions and on 7 of the admissions had acute respiratory infections diagnosed as bronchitis or bronchopneumonia. Rales were heard at the time of her third admission at 11 months, and they were noted with each subsequent entry until the time of her death. The leukocyte count usually ranged between 6000 and 7000 cells per cu. mm., but on one occasion totaled only 3800 with a differential count of 97 per cent lymphocytes. There was generally fever of 2 or 3 degrees F. Cough, however, was always a prominent feature. No specific exanthemata had occurred. The family history did not contribute to the diagnosis.

"At the age of 22 months, weighing 18 pounds 4 ounces, she was admitted with acute poliomyelitis which involved principally the lower extremities and the hip muscles. In 1945, a year later, she had surgical correction of a flexion deformity to the right lower extremity (Soutter operation), and it was for traction to this part that she last entered the hospital in April 1946.

"Physical examination. Physical examination revealed mild conjunctivitis and a moderate inflammation of the nasal passages and pharynx. The temperature was 102.4 F. rectally, the pulse rate 150 per minute and the respiratory rate 34 per minute. She weighed 21 pounds. She had a hoarse, paroxysmal cough accompanied by pain in the chest, most marked in the right lateral hemithorax. Rales were not noted at the initial examination, but a friction rub was thought to be present. The provisional diagnosis was acute respiratory infection, the type to be determined.

"Laboratory data. The initial leukocyte count was 8200, with a differential count of 65 per cent granulocytes and 35 per cent lymphocytes. The urinalysis was not remarkable. At entry, a swab of the throat, cultured on blood agar,

yielded an abundant growth of hemolytic streptococci in addition to the usual flora.

"Treatment and course. Her course in the hospital was disappointing from the beginning. On the second day many fine and medium rales were heard throughout the lung fields, the temperature increased to 103.2 F. and the cough became more distressing. A clinical diagnosis of bronchopneumonia was made. Penicillin was administered intramuscularly, 15,000 units every three hours, and a sedative cough mixture was prescribed. She did not improve. On the fifth day her temperature was 104.2 F., and she was moderately dyspneic. X-ray films of the lung fields revealed a uniform stippling (Fig. 1). The dosage of penicillin was increased to 20,000 units every three hours, and 5 grains of sulfadiazine were given orally every six hours. Because of mild cyanosis, oxygen was employed as required. On the following day she was transfused with 200 cc. of whole blood, and a moderate, temporary improvement followed.

"During the second week of her illness a maculopapular eruption appeared over the entire body surface and slowly faded after five days. Three weeks previously she had been intimately exposed to a child who developed measles, and on that day she was given 5 cc. of convalescent measles serum intramuscularly.

"By the third week her temperature was swinging from 99 to 103 or 104 F. each day, her cough was more troublesome, she resented being disturbed for care and her respiratory rate during sleep varied from 48 to 80 per minute. Weakness had become pronounced. Penicillin was increased to 50,000 units every three hours, and sulfadiazine was administered subcutaneously instead of orally without any clinical improvement. On her twenty-fifth hospital day she expired.

"Throughout her illness examinations of the chest revealed only an abundance of moist rales, greater in the left upper and right lower lung fields. No consolidations could be demonstrated with satisfaction. Cultures of blood, of catheterized urine and of spinal fluid were negative. The highest leukocyte count, the initial one, was 8200. The erythrocyte count and hemoglobin remained at satisfactory levels. The erythrocyte sedimentation rate three days before expiration was 1.5 mm. in one hour. One throat culture, taken midway in the course, showed a heavy growth of hemolytic *Staphylococcus aureus*; this and the hemolytic *Streptococcus* were the only significant organisms cultured. Although an intradermal tuberculin test in a dilution of 1:10,000 was negative, studies relative to tuberculosis were in process at the time of the patient's death."

DISCUSSION OF CLINICAL DATA

Dr. R. O. Sherberg. "I just want to add that tuberculin patch tests had been done during the past year because of the frequent recurrences of cough. These were negative. Streptomycin had been requested through Dr. Chester Keefer, but was not available for the organisms we had recovered."

Dr. J. J. Waring. "It seems to me that a very important symptom in the history is the story of repeated attacks of what appears to be bronchitis or bronchopneumonia. That always raises the question: What causes the repeated

attacks? I should like to mention one or two possible causes as they occur to me. There is some possibility that this may be tuberculosis. In children, if tuberculosis is sufficient to cause considerable fever, they usually run a downhill course, rather than recover, as this child did on several occasions. Nevertheless, I believe you have to prove that the child does not have tuberculosis.

"The second possibility is that the lung is constantly being reinfected or irritated by something present in the lung itself. One of the sources for such chronic irritation is the instillation of oily material into the nose. It is entirely possible that the child can have serious trouble in the nature of a lipoid pneumonia due to oily nose drops.

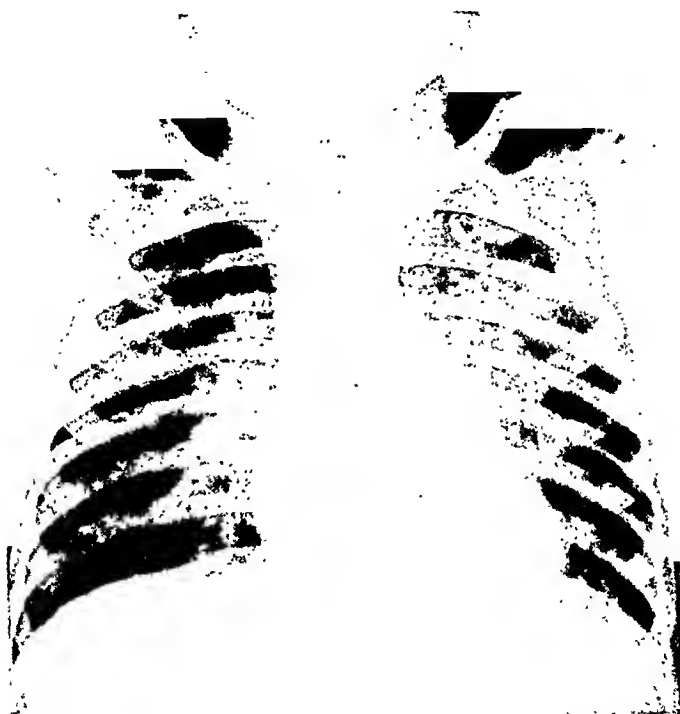


FIG. 1. X-RAY FILM, TAKEN WITH PORTABLE APPARATUS, SHOWING WIDESPREAD STIPPLING OF THE LUNG FIELDS

"There is a third possibility. We had in this town some years ago a fine young doctor who, over a period of fifteen to twenty years, had at least a dozen attacks of pneumonia in the same lung. They always pursued very much the same course. It wasn't recognized until his last attack that he had an esophageal-bronchial fistula, and this was diagnosed by the roentgenologist. Therefore, I would raise the question of tuberculosis, the presence of a foreign body (oil), or a fistula between the esophagus and some part of the respiratory tract. In addition one must also think of the miscellaneous virus infections."

Dr. F. B. Stephenson. "An x-ray film of the chest, taken March 31, 1944, or about two years prior to the present episode, shows nothing abnormal. The films made during her final illness show very distinct bronchopneumonic changes. In addition there is a generalized overcasting, and within that there are many

densities which are not discrete and round like those usually seen in typical cases of miliary tuberculosis.

"A good many atypical, or virus, pneumonias are seen in children. Gradually, I have acquired a very distinct impression that in nearly all cases the process is localized to some particular segment of the lung. This is unlike ordinary types of pneumonia which are more likely to be widely distributed in combination with atelectasis and bronchopneumonia. The infection in this particular case seems to involve certain segments of the bronchial tree. In many places you will see adjacent parenchymal clouding which suggests peribronchial inflammation, tending to give the conglomerate picture of parenchymal infiltration and not the dense lesion one gets with consolidation. In this film many of the primary lobules are outlined, which may be an interstitial inflammatory condition. I believe this looks very much like some of the cases of virus infection we have seen here and makes me think of a segmental pneumonia.

"It has been shown rather conclusively that virus pneumonias are segmental and that the bronchial branch which supplies the involved area is found at autopsy to be surrounded by inflammatory changes. I have reached the conclusion that virus pneumonia is bronchial-borne, producing primary bronchitis with secondary parenchymatous change, and that is what we see in the x-ray film."

Dr. H. H. Gordon. "I think if one limits his attention to the history, the story of a child with infection from the age of 9 months, with repeated bouts of bronchitis or bronchopneumonia, he should consider three possibilities: (1) the possible aspiration of oil with secondary mild flare-ups; (2) the possibility that atelectasis developed in some part of the lung which never fully cleared up, the lung becoming subject to reinfection and thus developing bronchiectasis; and (3) the possibility that the repeated respiratory infections were associated with fibrocystic disease of the pancreas. Such an association sometimes occurs in the first year of life. This diagnosis can, I think, be dropped in this case, however, as there is no good support for it in the history. Fitting with the diagnosis of lipoid pneumonia as the underlying lesion is the fact that the flare-ups were relatively mild and not associated with marked increase in the white blood cell count. In the final illness, however, she apparently had enough infection to warrant a rise of temperature to 103 F. It would be nice if we knew more from a bacteriologic standpoint, but that is difficult with young children. We are able to get throat cultures, but information which might be gained from a sputum examination is lacking."

Dr. W. W. Jones. "Was the rash due to measles?"

Dr. Sherberg. "She had been exposed and had been given immune serum. I believe she did have measles. Certainly it looked like it."

Dr. C. J. Stettinheimer. "Measles can often complicate tuberculosis and produce a picture just like this. The resistance is lowered, and a miliary spread follows the attack of measles. It should be stressed, too, that in infants and young children with hemolytic streptococcal and staphylococcal infections we have seen associated disturbances in the gastrointestinal tract, and also septicemia with



FIG. 2. Esophagus opened longitudinally. Note accentuation of rugae, cross-hatching and beading.



FIG. 3. Cross-section of lung. Note prominence of the peribronchial tissues and small areas of consolidation.

multiple abscesses throughout the lungs, probably seeded by the blood stream infection. This child had a heavy growth of these organisms in the throat cultures."

Dr. Jones. "Does the picture of lipid pneumonia have a distinct pattern?"

Dr. Stephenson. "Yes, but I think this is more of an inflammatory interstitial pneumonia. However, lipid pneumonia cannot be entirely ruled out since it may be widely distributed."

Dr. Waring. "Lipid pneumonia in the adult is prone to involve the right lower lobe. The physical signs of consolidation are elicited. The x-ray evidence of the lesion takes the form of quite dense areas of opacity."

NECROPSY FINDINGS

Presented by Dr. Harold D. Palmer

"This 3½ year old girl came to necropsy in a relatively well nourished state. External development was normal. The body was extremely livid and was without evidence of edema or jaundice, was not rigid and was apparently in a state of normal hydration.

"*Internal examination.* The positive findings only are mentioned. The primary pathologic conditions were found in the chest. The pleural cavities were free of fluid but there were scattered fibrous adhesions of delicate structure between the visceral and parietal pleura on the left side. The chest viscera were removed *en bloc*. The esophagus was found moderately dilated in its lower third. Opened longitudinally, it revealed very prominent longitudinal mucosal folds of yellowish color, cross-hatched in a manner that gave them a beaded appearance (Fig. 2). The mucosa had lost its velvety appearance, and the folds appeared almost scaly. The surface was cracked between the longitudinal folds. This involvement was most pronounced in the lower third, faded gradually superiorly and ended abruptly at the cardia. The thymus gland weighed 5 Gm. and had a normal appearance. The heart was moderately dilated, weighed 68 Gm. and was very flabby. The great vessels were normal, and particular study was made of the pulmonary artery and its branches. A number of enlarged lymph nodes were found in the superior mediastinum adjacent to the thymus, at the bifurcation of the trachea, in the lung hili and in the posterior mediastinum along the esophagus. They measured up to 2 cm. in diameter, had tense capsules and presented opaque, gray, moist cut surfaces. The mucous membrane of the trachea was reddened. The primary bronchi appeared to be acutely inflamed and contained thick, gray, mucoid material. From the smaller bronchi almost white cylindrical casts could be expressed. The lungs were full in volume, felt shotty and were very heavy, weighing 229 and 203 Gm. In spite of their weight they floated on water. The cut surfaces showed the prominent peribronchial markings of interstitial pneumonitis with grayish red areas of apparent consolidation about them (Fig. 3). Areas of hyperventilated parenchyma were seen between these patches. The dome of the diaphragm was placed at the level of the fifth rib on the right and of the sixth rib on the left. The liver edge was located 9 cm. below the right costal margin in the mid-clavicular line. This was due to the low position of the diaphragm and to enlargement caused by congestion and fatty metamorphosis of moderate degree (confirmed microscopically). There were scattered adhesions of cobweb type be-

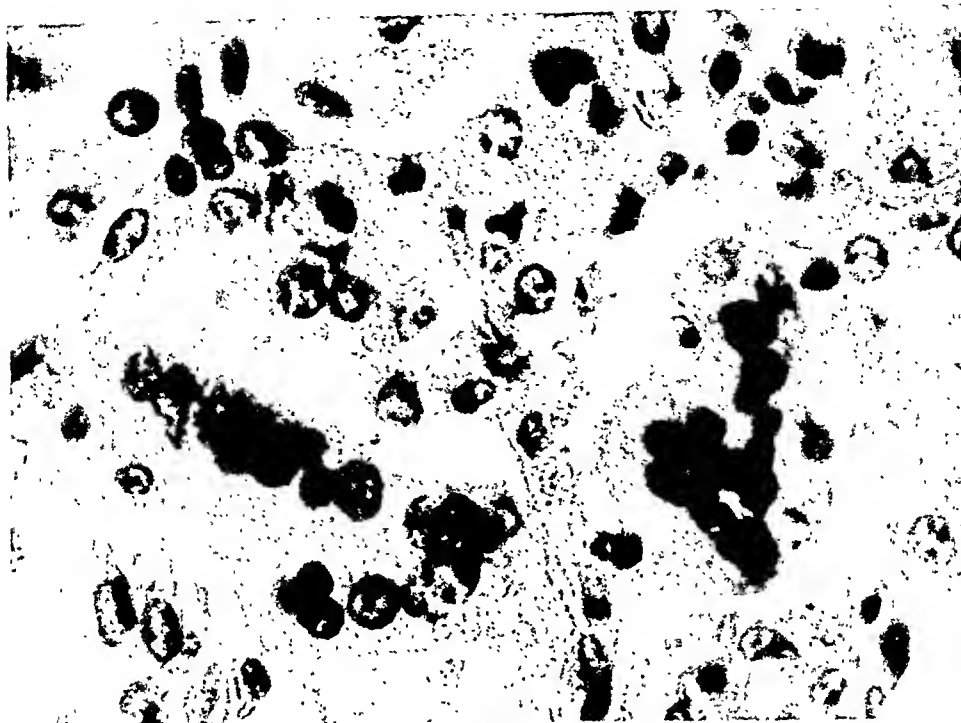


FIG. 4. Section of lung. One giant cell lies free in an alveolus; another is forming in the septal wall. $\times 1032$.

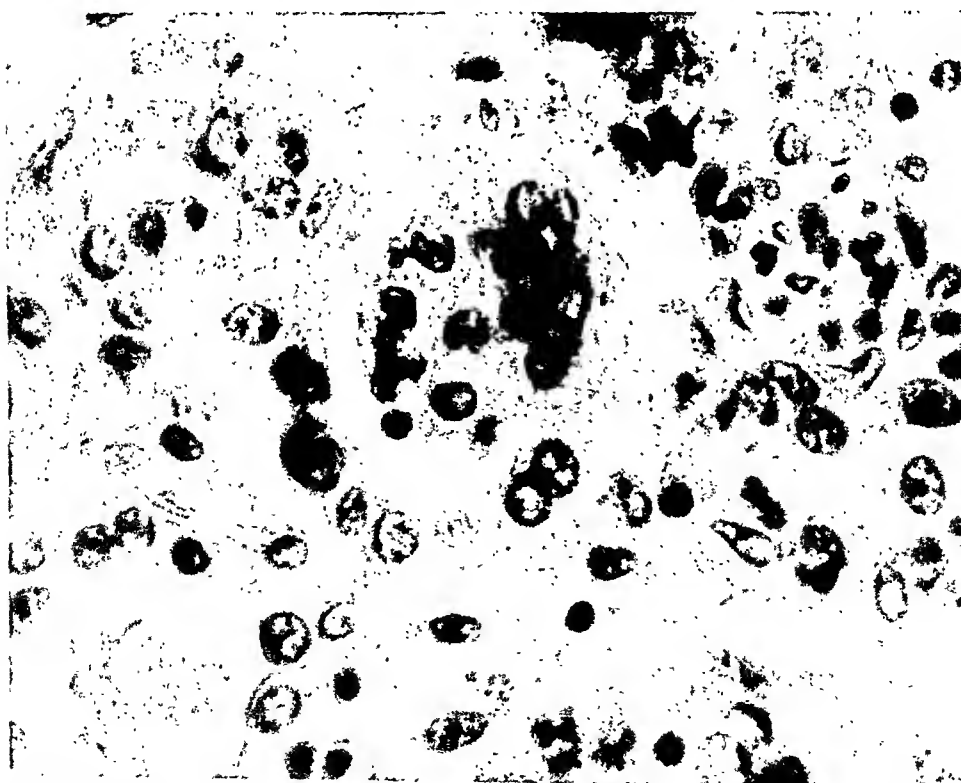


FIG. 5. Section of lung. Note change in septal cells leading to giant cell formation. $\times 1032$.

tween the liver and the diaphragm and between the spleen and the diaphragm. The spleen was steel blue in color and firm, weighed 40 Gm. and histologically presented reticulo-endothelial hyperplasia and polynuclear infiltration of the pulp. Except for engorgement and cloudy swelling of parenchymatous elements, no changes were noted in the other abdominal or pelvic organs. The pancreas was entirely normal.

"Bacteriologic examinations were made of the lungs and mediastinal lymph nodes. A pure culture of *Pneumococcus* was obtained from the lungs and tests with specific serums revealed capsular swelling. The same organism and scattered colonies of *Staphylococcus aureus* were recovered from the lymph nodes.

"A revelation came with histologic examination of the lungs. Under low power in any of the sections one immediately noticed a profusion of giant cells (Fig. 4). It was seen that these were not developed with lesions that had any of the histologic features of tuberculosis. The giant cells were found in the air sacs or terminal air ducts and often seemed to line these structures, arising apparently by proliferation and fusion of septal cells (Figs. 4 and 5). Many of them contained as many as 35 nuclei. The cytoplasm was stained with eosin, the nuclei were vesicular and, in the older elements, piled in the center of the cell. The giant cells that were in the process of formation still had nuclei that were confined to the original boundaries of the individual cells, the cytoplasm of which was fusing (Figs. 4 and 5). In places the proliferations of the septal cells bulged as small masses into the air sacs (Fig. 5). In addition the epithelium of the entire tracheobronchial tract had undergone squamous metaplasia to a marked degree (Fig. 6). Not a single section showed absence of this change. Within the giant cells and the bronchial epithelium numerous inclusion bodies were demonstrated. These were both intranuclear (Fig. 7) and cytoplasmic in location, varied from 2 to 7 microns in diameter and were often surrounded by a halo. When in the cytoplasm, they were often close to, and caused indentation of, a nucleus. In sections stained with hematoxylin and eosin they were eosinophilic, but took the stain with non-uniform intensity. Some S_3 stains (a modified Masson trichrome stain adapted for the demonstration of inclusion bodies³) were made by Dr. Charles L. Davis of the United States Bureau of Animal Industry. The inclusions were present in enormous numbers, often outnumbering the nuclei of the giant cells. The intranuclear and cytoplasmic inclusions were identical. Inclusion bodies were found only in the lungs. Many of the bronchioles contained thick mucin with cellular detritus, including a few remnants of giant cells. The peribronchial mucous glands were dilated, and the acini contained inspissated secretion. This part of the picture is similar to that seen in the lungs in cystic fibrosis of the pancreas. The interstitial tissue radiating from the bronchioles was much widened and presented an active pneumonitis. Large and small mononuclear cells with a few polymorphonuclears made up the cellular exudate in the interstitial tissue.

"The esophagus presented great thickening of the epithelial layer with a wide, keratinized surface layer. The subepithelial layer was infiltrated with lymphocytes, and there were minor breaks in the epithelial layer in the depth of mucosal folds. The lesion in the esophagus suggested leukoplakia.

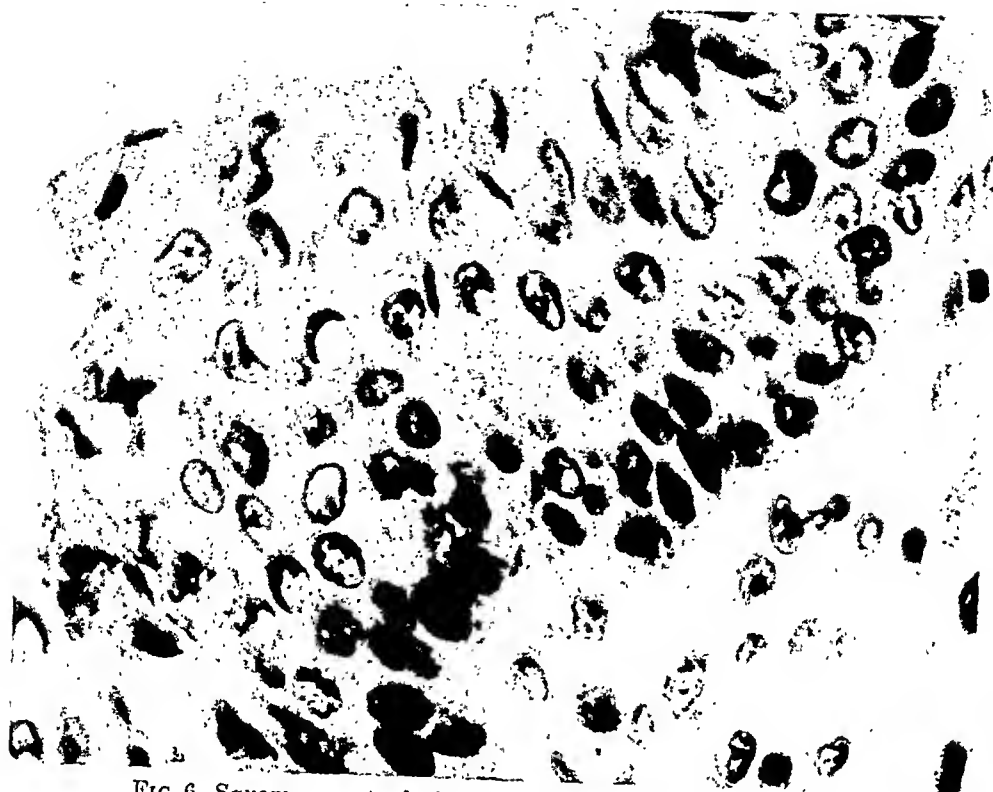


FIG. 6. Squamous metaplasia of bronchial epithelium. $\times 1032$.

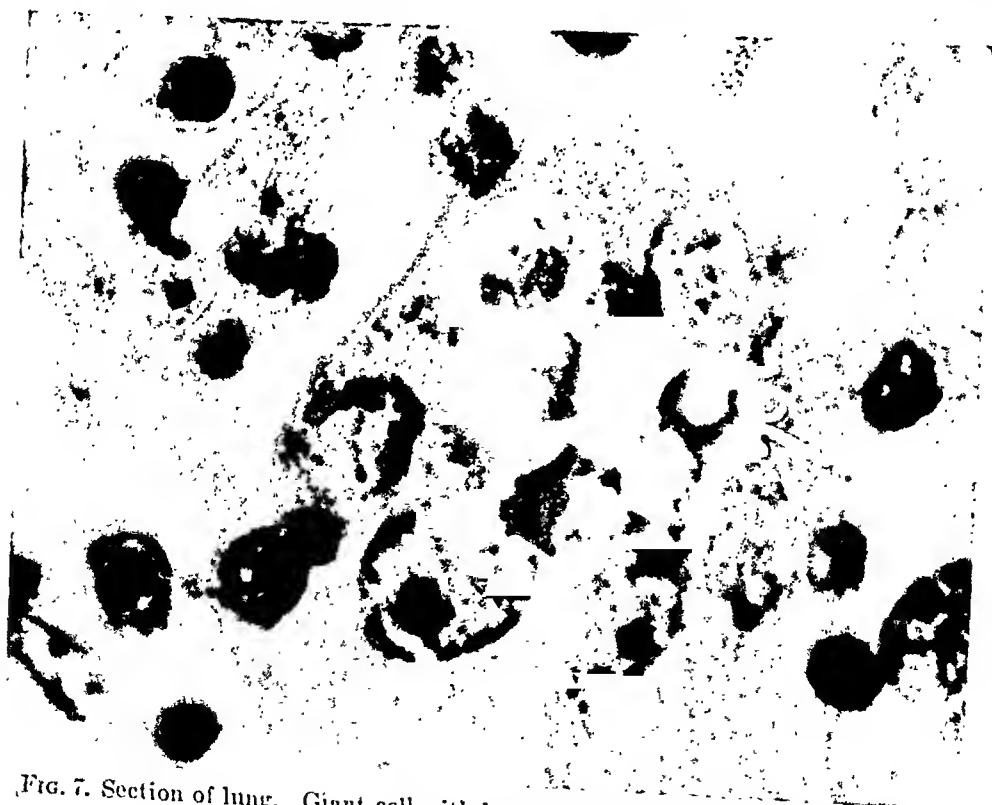


FIG. 7. Section of lung. Giant cell with intranuclear inclusion (arrow). $\times 2280$.

"Ziehl-Neelsen stains were negative for acid-fast organisms, and Gram stains revealed occasional Gram-positive football-shaped diplococci in both the lungs and the lymph nodes.

"The conclusion was that this was a case of giant cell pneumonia of Hecht."

DISCUSSION OF PATHOLOGIC FINDINGS

Dr. Palmer. "Giant cell pneumonia is an interstitial pneumonitis of infants and young children. In the absence of superimposed bacterial infection, it runs a subacute or chronic course which clinically, and with present laboratory methods, is indistinguishable from other forms of subacute or chronic infections of the lungs in this age group.

"It was first described by Hecht² in 1910. He reported 27 cases among a group of 167 infants with subacute or chronic pneumonia coming to necropsy. In spite of this suggested high incidence in Germany, relatively few cases have been reported since 1910. It is probable that a much greater number of cases has been recognized than has been reported. Giant cell pneumonia is reported most frequently as a part of the pathologic changes in the lungs in fatal measles. It has been reported in syphilitic and tuberculous infants. It has also been described as an independent lesion.

"In 1939 Chown¹ interpreted the lesion to be the result of vitamin A deficiency. He was the first to suggest that the disease is a definite pathologic entity. Pinkerton, Smiley and Anderson,⁴ although discarding the suggestion that vitamin A is the principal etiologic factor, added important evidence that the disease is an entity. They were the first to describe the inclusion bodies which are present in the alveolar lining cells, giant cells and bronchial epithelium of affected lungs. The same authors pointed out the similarity in numbers, character and intracellular position of the inclusion bodies of giant cell pneumonia to those of distemper in animals. They leave the suggestion that giant cell pneumonia, occurring independently of measles, may represent distemper in man.

"The name giant cell pneumonia is not descriptive since the lesion is an interstitial pneumonitis accompanied by a proliferation of septal cells to form giant cells which often line the alveoli, and a proliferation of the bronchiolar and alveolar duct epithelial cells to form either giant cells or a metaplastic epithelial lining of these structures. The evidence that giant cell pneumonia is the lesion of a specific virus or group of viruses is not yet conclusive since a virus has not yet been recovered from the diseased tissues. Circumstantial evidence, however, is abundant. The occurrence of inclusion bodies in great numbers within the nuclei and the cytoplasm of the giant cells and the bronchial tract epithelium is striking. The fact that these inclusion bodies are identical in morphology in the two locations is also important. The similarity in structure and distribution of these inclusion bodies with those found in the lesions of distemper of animals has been carefully described by Pinkerton *et al.*"

CLINICOPATHOLOGIC CORRELATION

Dr. Palmer. "This infant suffered from the age of 9 months with chronic bronchopulmonary disease. The course became acute only during the last twenty

days of life. In consideration of the bacteriologic result along with the histopathologic findings, it is believed that the acute picture is the result of a superimposed pneumococcal infection. None of the findings in the lungs is typical of primary pneumococcal infection at this age. The histopathology of the lymph nodes, however, is quite typical of bacterial lymphadenitis. The importance of the virus of rubcola in the pathogenesis of the lesions in the lungs is also difficult to assess. It is of interest, however, that the rubcola developed in this case only after evidence of pulmonary disease had been manifest for many months and after mottling of the lung fields was demonstrated in the x-ray films. It is also important to note that the inclusion bodies were found in great numbers in intranuclear as well as in intracytoplasmic location and that this coincides with Pinkerton's observation in the mink and also in lungs of persons who have not been associated with measles. It is also true that the inclusion bodies which are found in the giant cell pneumonia complicating measles are described as intracytoplasmic in location and are rarely present in great numbers as in this case. Giant cells were absent in the lymph nodes.

"Our patient exhibited all of the features of giant cell pneumonia described by the authors. In addition, she presented very severe changes of a metaplastic nature in the epithelium of the esophagus. The character, number and intracellular location of the inclusion bodies are the same as described by Pinkerton. Although the patient experienced modified measles, this development came after the lung changes were manifested clinically and roentgenologically."

Dr. Enid Rullege. "Do you think that the squamous cell metaplasia may have been going on for a long time? Could it be on a vitamin A deficiency basis?"

Dr. Palmer. "Squamous cell metaplasia in the bronchial tract was thoroughly established as a manifestation of vitamin A deficiency by Wolbach.⁵ The question of vitamin A deficiency in relation to metaplasia in this disease has not been thoroughly studied. Pinkerton *et al.* discuss this problem and suggest that the virus may so alter the epithelial cells that they are unable to utilize vitamin A. These authors also state that many of the clinical features of distemper resemble those of vitamin A deficiency. Hyperkeratinization of the skin of the paws (in dogs) and the occurrence of pustular lesions in the hair follicles are definitely suggestive of vitamin A deficiency."

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EDITORIAL

EXAMINATION OF THE ORAL CAVITY IN PERFORMANCE OF ROUTINE AUTOPSIES*

The pathologist, usually a thorough and tireless student, strives to learn all phases of disease processes, their pathogenesis, their anatomic, physiologic and biochemical alterations as well as the correlations between pathologic processes in one system and alterations occurring in various other systems of the complex organism. He himself will be the first to admit his usual neglect of the oral region, the most accessible part of the gastrointestinal tract.

The general pathologist can contribute much to our knowledge in oral pathology. Let us take an example. In the dental field the most important problems, of course, are dental caries and diseases that alter the integrity of the supporting structure of the teeth. Teeth may be saved from the ravages of caries only to be lost through disintegration of their normal support. Numerous and elaborate theories (with loopholes) have been propounded to explain periodontoclasia, but none can be accepted or completely discarded in view of our present lack of knowledge. At the present time no one can say with certainty whether alveolar bone, connective tissue or epithelium is first affected. Until this is determined, one must grope in the dark, seeking the basic factors in the pathogenesis of periodontoclasia.

The mechanical difficulties of such studies surely are not insurmountable. A simple technic, which is not time-consuming, could be worked out whereby tissues for study could be obtained without disfigurement of the body. It does seem that much necessary progress could be made if such studies were made routinely.

Sections from some portion of the dental arch of persons coming to autopsy, as well as sections from other tissues such as the tongue and buccal mucosa, should be taken routinely, for in no other way can proper correlations be made between manifestations of disease of the mouth and of other parts of the body.

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HISTOPATHOLOGIC EFFECTS OF NITROGEN MUSTARD THERAPY UPON NORMAL AND NEOPLASTIC HEMATOPOIETIC TISSUES*

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INTRODUCTION

Since the introduction of nitrogen mustard therapy in 1942, there has been widespread interest in the clinical response which may be induced in neoplasms of the hematopoietic tissues. This study is an attempt to evaluate the histopathologic changes produced by one of these compounds, methyl-bis (β -chloroethyl)amine hydrochloride, in the bone marrow and neoplastic tissue and to determine, if possible, the reasons for variations in clinical response.

The clinical results of this type of therapy have been discussed in detail by Goodman *et al.*,⁶ Jacobson *et al.*,¹¹ Spurr *et al.*,²² and Karnofsky *et al.*¹² In general they have been similar to those seen following roentgen irradiation. The clinical response may vary from no remission or a negligible remission lasting a week or two to one lasting twenty-four months. The duration of response varies to a great extent with the type of tumor. The reticulum cell sarcoma, the sarcomatous type of Hodgkin's disease and multiple myeloma show brief or no remissions, while lymphosarcoma and Hodgkin's granuloma have more prolonged remissions.

The effects of the methyl-bis (β -chloroethyl)amine hydrochloride are not selective against malignant tissues, and there is a constant damage to the bone marrow with each course of therapy. The effects of the compound, as studied by smears of aspirated sternal marrow and peripheral blood, have been considered by Jacobson *et al.*,¹¹ and Spurr *et al.*²² in adopting the standard dose of 0.1 mg. per kilogram of body weight, given intravenously on each of four successive days. With this dose there is usually produced, in the third week after treatment, a leukopenia of 2000 to 3000 leukocytes per cu. mm. which generally lasts less than a week. Depression of the platelets and erythrocytes may develop, but is usually insignificant.

MATERIALS AND METHODS

Several techniques were applied to obtain tissues for biopsy. Many of the tissues were obtained by repeatedly puncturing the same lymph node or the spleen by means of the Vim-Silverman needle under local anesthesia. This was the most satisfactory method of study since it enabled one to study the same node, an important consideration in Hodgkin's disease where the nodes of any one patient at one time may vary so much.

* This work was supported, in part, by the American Cancer Society on recommendation of the Committee on Growth of the National Research Council. Presented at the Twenty-Sixth Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 28, 1947. Received for publication, March 2, 1948.

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Repeated surgical excision of lymph nodes for biopsy was also employed, but this technic was less suitable than the needle technic for obtaining tissue for biopsy since it made serial study of the same lymph node impossible.

Another method attempted was to expose a lymph node and remove a thin slice of tissue from its surface. The overlying skin was drawn over the node by retention sutures. Later, at selected intervals, the sutures were loosened and another slice taken from the surface of the node. Unfortunately, this technic introduced inflammatory changes which interfered with the evaluation of the sections.

Bone marrow for biopsy was obtained by aspiration from the sternum, using a 15 gauge needle $1\frac{1}{2}$ inches long with a flattened bevel.

At first the tissues were fixed in Zenker-formol for approximately three hours at room temperature. This did not fix the neutrophil granules, and sharper staining of these granules was obtained after fixation for eight hours. After embedding in nitrocellulose, the sections were cut serially first at 8 microns, later at 6 microns. The slides were stained with hematoxylin eosin-azure II. Occasional slides were impregnated with silver to demonstrate reticular fibers.

OBSERVATIONS

Although there is no difficulty in finding microscopic evidence of the cytotoxic effects resulting from the nitrogen mustards in the experimental animal, when using a median lethal or lethal dose (Kindred,¹³ Block²), it is often difficult to demonstrate histopathologic effects in man, in the dose used clinically. This is especially true of the lymph nodes and to a lesser extent of the bone marrow.

I. Hodgkin's Disease

A. Marrow. The marrow biopsies were obtained from 9 patients at intervals from one to eighty-eight days after the initial injection of nitrogen mustard. The chronologic distribution of the biopsies is shown in Table 1.

The hypercellularity in the marrows before treatment was due primarily to a myeloid hyperplasia. Practically every patient had an absolute increase in the number of eosinophils and eosinophil precursors. None of the biopsies revealed the specific lymphogranulomatous lesion of Hodgkin's disease (Fig. 1).

The changes seen within twenty-four hours after any single injection consisted of a slight increase in the number of degenerating cells (Fig. 2). Only one patient, from whom marrow was removed one and four days after the first injection, failed to show a significant increase in the number of degenerating cells. However, because of the paucity of degenerating cells, it was not possible to determine which cell type was most susceptible. It was our impression that there was usually no change in the number of mitoses, although occasionally it seemed that the number was decreased.

A phase of phagocytic activity corresponding to that seen in the experimental animal at higher doses (Kindred,¹³ Block²) was not observed, probably due to the lack of sufficient degeneration to incite a phagocytosis that could be noted microscopically.

TABLE 1

INTERVALS OF BIOPSIES FROM BONE MARROW IN 9 PATIENTS WITH HODGKIN'S DISEASE, SHOWING PREVIOUS TREATMENT AND RESPONSE AND PRESENT TREATMENT WITH NITROGEN MUSTARD

PATIENT, SEX, AGE	DURATION OF DISEASE	PREVIOUS THERAPY; RESPONSE, DURATION		TOTAL DOSE; RESPONSE, DURATION	DAYS BETWEEN INITIAL INJECTION AND BIOPSIES (DOSE IN PARENTHESES PRECEDING BIOPSY)*
		Röntgen Ray	Nitrogen Mustard		
G. W.† M 22	24 mo.	13,000 r to pelvis; fair, 3 months	None	28 mg.; good, 6 months	½(7 mg.)
S. G. F 19	14 mo.	3300 r to mediasti- num, 300 r to neck; good, 3 mo.	None	20 mg.; good, 2½ months	1(5 mg.), 4(20 mg.)
E. S. F 27	24 mo.	To regional nodes; good, 6 months	None	24 mg.; good, 5 months	3(18 mg.), 7(24 mg.)
M. D. F 28	12 mo.	None	One course; good, 5 mo.	24 mg.; good, 5 months	4(24 mg.), 9, 17, 24, 31
H. H. F 31	24 mo.	700 r to mediasti- num, 700 r to axillae, 500 r to groin; good, 6 months	Two courses; good, 3 mo.	20 mg.; good, 4 months	8(20 mg.), 16, 22, 56
J. H. M 31	10 yrs.	Extensive; good with decreasing remissions	One course; good, 4 mo.	28 mg.; good, 4 months	12(28 mg.), 35, 49
W. B. M 31	30 mo.	Extensive; short remissions	None	21 mg.; good, 4 months	28(21 mg.)
E. M. F 32	30 mo.	Extensive; de- creasing remis- sions	None	60 mg.;‡ good, 4 months	30(60 mg.)
E. G. F 33	24 mo.	None	None	28 mg.; good, 3 months	SS

* A course of treatment consisted of 0.1 mg./Kg. body weight of HN₂ intravenously in 4 consecutive daily doses.

† Sarcoma of prostate.

‡ Consisted of a single dose of 30 mg. plus 5 mg. given six times.

The primary cytotoxic phase was followed by an atrophic phase, lasting from about the eighth day until the onset of regeneration at the fifteenth to twentieth day. This atrophic phase was usually quite clear-cut in all the patients. The atrophy was due primarily to a decrease in neutrophil and eosinophil precursors.

TABLE 2
INTERVALS OF LYMPH NODE BIOPSIES IN 6 PATIENTS WITH GRANULOMATOUS TYPE
HODGKIN'S DISEASE, SHOWING PREVIOUS TREATMENT AND RESPONSE AND
PRESENT TREATMENT WITH NITROGEN MUSTARD

PATIENT, SEX, AGE	DURATION OF DISEASE	PREVIOUS THERAPY; RESPONSE, DURATION		TOTAL DOSE; RESPONSE, DURATION	DAYS BETWEEN INITIAL INJECTION AND BIOPSIES (DOSE IN PARENTHESES PRECEDING BIOPSY)
		Roentgen Ray	Nitrogen Mustard		
C. C. M 23	30 mo.	1400 r to neck; good, 6 months	6 courses;* good, 2 to 4 months	12 mg.;† poor, 1½ months	½(6 mg.)
J. B. M 26	12 mo.	None	None	28 mg.; poor, 1½ months	1(7 mg.), 2(14 mg.), 3(21 mg.), 4(28 mg.)
J. B. M 26	12 mo.	None	One course; poor, 1½ mo.	30 mg.; none, 0 months	2(12 mg.)
T. L. M 43	16 mo.	1290 r to right in- guinal, 1510 r to left inguinal, 300 r to lumbar; good, 4 months	None	26 mg.; fair, 2 months	4(26 mg.)
J. W. M 30	20 mo.	One extensive course 4600 r; good, 4 mo.	None	26 mg.; fair, 2½ months	10(26 mg.)
E. M. F 32	30 mo.	Extensive decreas- ing remissions	None	60 mg.;‡ good, 4 months	30(60 mg.)

* Course of therapy was the same as in Table 1.

† Incomplete course, only two injections.

‡ Same course as described for this patient in Table 1.

It preceded closely the leukopenic phase in the peripheral blood. The plasma cells never showed a decrease in number and in several instances seemed to be increased. The erythroblasts were either slightly decreased or unchanged in number (Fig. 3).

In the regenerative phase (Fig. 4), the marrows for the next few months were always as active and well-populated as the marrows before treatment. In some of the patients an increased activity was seen, due usually to an increase in granulopoiesis, often with a preponderance of immature forms. Mitoses were generally more numerous in this regenerative phase than before treatment. Overactive marrow was sometimes seen as late as three or four months after treatment.

One patient in this series received a total of 60 mg. in seven consecutive days, including one single dose of 30 mg. This patient developed a leukopenia of 500

TABLE 3
INTERVALS OF LYMPH NODE BIOPSIES IN FOUR PATIENTS WITH SARCOMATOUS TYPE OF HODGKIN'S DISEASE SHOWING PREVIOUS TREATMENT AND RESPONSE AND PRESENT TREATMENT WITH NITROGEN MUSTARD

PATIENT, SEX, AGE	DURATION OF DISEASE	PREVIOUS THERAPY; RESPONSE, DURATION		TOTAL DOSE; RESPONSE, DURATION	DAYS BETWEEN INITIAL INJECTION AND BIOPSIES (DOSE IN PARENTHESES PRECEDING BIOPSY)
		Roentgen Ray	Nitrogen Mustard		
R. Y. M 24	15 mo.	Extensive; brief re- missions	None	26 mg.; poor, 1½ months	2(13 mg.), 3(19.5 mg.)
J. A. F 19	12 mo.	None	One course; poor, 2 months	28 mg.; good, 11 months	3(14 mg.)
L. W. F 24	12 mo.	Extensive; de- creasing remis- sion	Two courses; good, 2 to 3 mo.	28 mg.; poor, 2 months	4(28 mg.), 8
B. D. F 37	36 mo.	Extensive; de- creasing remis- sions	None	36.6 mg.; poor, 2 months	11(36.6 mg.)

cells during the fourth week after treatment, the count returning to normal at about twelve weeks after treatment. Her biopsy before treatment (Fig. 1) revealed the hyperactive marrow with myeloid hyperplasia usually seen in Hodgkin's disease. Her biopsy after treatment (Fig. 5), thirty days after the first injection, revealed extreme atrophy with myxomatous degeneration. There was a diffuse sprinkling of plasma cells, hemocytoblasts and inflammatory polyblasts. Occasional small foci of erythroblasts were seen. The marrow was similar to that seen in experimental animals subjected to a median lethal dose of x-ray to the entire body or of nitrogen mustard. At autopsy about ten months later regeneration was complete.

B. Lymph nodes. We classified the nodes into the paraganulomatous, granulomatous and sarcomatous types, according to the criteria of Jackson and

Parker.¹⁰ However, since the first two types reacted similarly, they will be referred to collectively as the lymphocyte-rich or granulomatous type. The variability of the nodes in Hodgkin's disease added to the difficulties of histologic analysis and contributed to conservatism in reaching conclusions. In many patients a much greater change was noted between biopsies of tissue obtained several months apart than between biopsies of tissue taken before and after treatment. Without biopsy before treatment one would have been inclined, as has been done repeatedly in the past in the study of irradiation effects, to ascribe this change to treatment rather than to the changing nature of the disease. (See reviews by Prym²¹ and Lubarsch and Wätjen.¹⁵) The histologic appearance of the nodes in any patient tended to change from a Hodgkin's granuloma to a Hodgkin's sarcoma. Further study is needed to clarify this point.

The effects of nitrogen mustard at the standard dose level were studied in four patients, one of whom received two courses of therapy for the granulomatous type of Hodgkin's disease (Table 2). One patient (C. C.) received only two injections of 0.1 mg. per kilogram. However, since tissue from this patient was obtained for biopsy twelve hours after the first injection, the changes noted would be comparable to those expected after a corresponding interval in patients who received a full course of four injections of 0.1 mg. per kilogram of body weight.

The changes produced by this level of dosage were very slight when the corresponding nodes were compared before and after treatment. During the twenty-four hours after any injection a very slight swelling of nuclei and an increase in cellular degeneration were found. This increased degeneration involved the lymphocytes, granulocytes and Sternberg-Reed cells, but not the reticular cells.

At four days after the initial injection there seemed to be slightly fewer mitoses, more degeneration and more swollen bizarre nuclei. At ten days the node appeared to have fewer lymphocytes than did the pre-treatment control (Fig. 8 *vs.* Fig. 7). But the differences between the nodes before treatment and after treatment were as extensive as one would have anticipated from the decrease in size of the nodes noted clinically.

The patient mentioned above who had an atrophic gelatinous marrow (Fig. 5) following a total dosage of 60 mg., had a node removed for biopsy thirty days after the initial injection of nitrogen mustard. There was a generalized atrophy of lymphatic tissue, and the excised node was only 1 mm. in diameter. However, it was moderately cellular with lymphocytes (Fig. 6), suggesting that a regenerative phase had been attained. In this patient the lymphatic regeneration was apparent when the bone marrow was atrophic. It seems, in this instance at least, that regeneration in the neoplastic lymph node was already well advanced

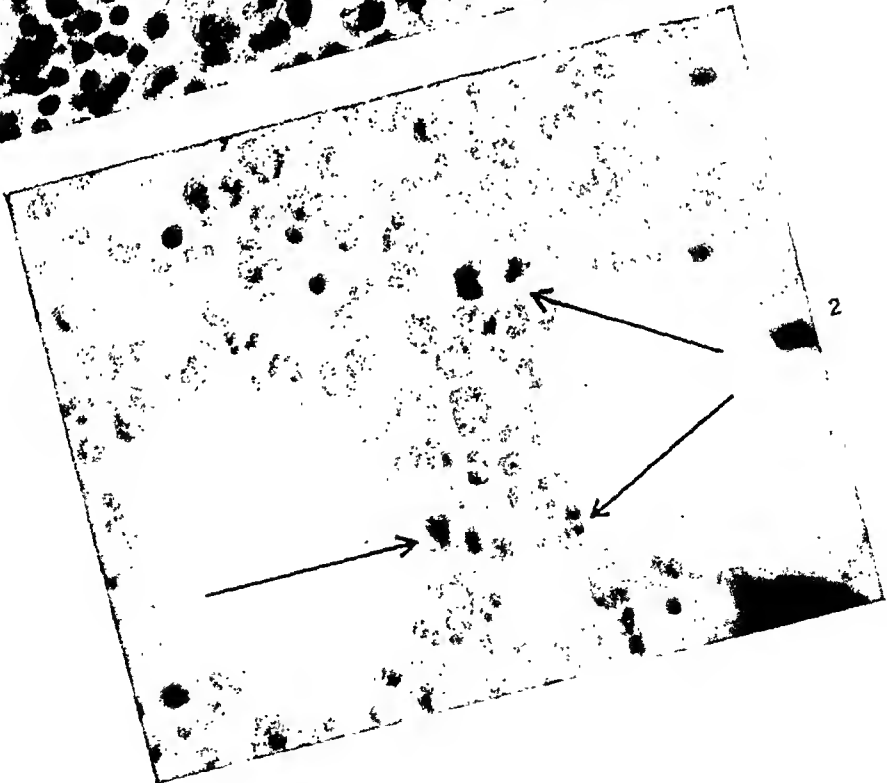
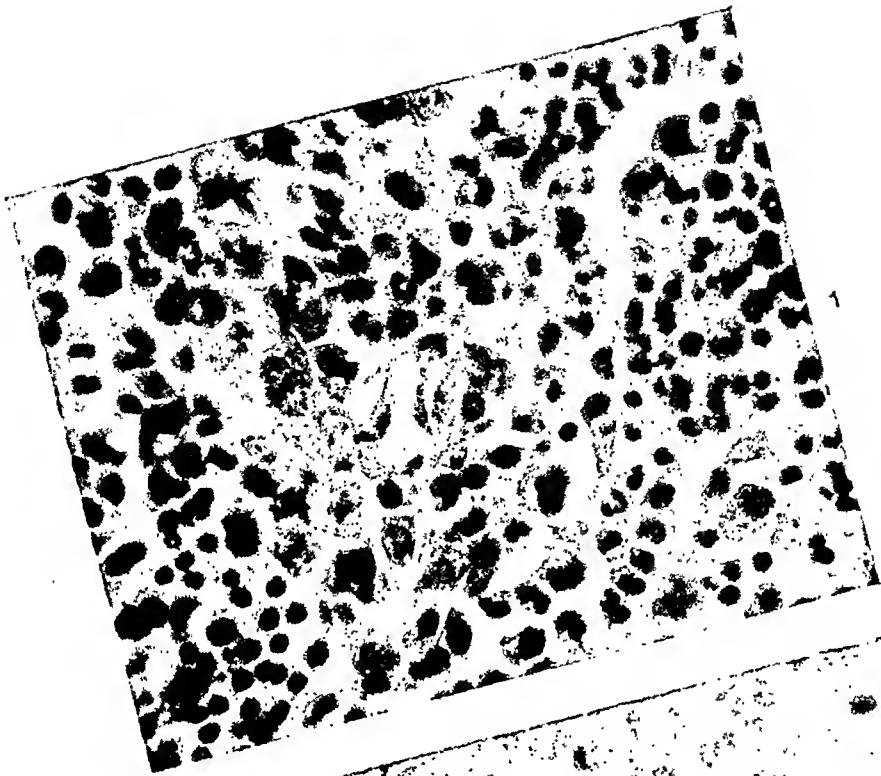
(All illustrations are from sections stained with hematoxylin eosin-azure II and photographed at a magnification of 700.)

FIG. 1. E. M., female, 32. Bone marrow before treatment.

FIG. 2. E. S., female, 27. Bone marrow twenty-four hours after second injection of 0.1 mg. nitrogen mustard per Kg. Arrow points to degenerating cells.

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at thirty days in spite of a dosage which was more than double that usually given and at a time when the bone marrow, which was not the site of the Hodgkin's disease, was still atrophic. Several months later biopsy of another node revealed a sheet of large lymphocytes and reticular cells.

The results in the sarcomatous type of nodes were less marked than those in the paraganuloma-granuloma types (Table 3). Clinically, three of these four patients had only a transitory softening of the nodes with a brief remission. Tissue from five nodes was obtained by the needle technic for biopsy from two to eleven days after the first injection. It is our opinion that during the acute phase, within twenty-four hours after any injection, there was a slight edema, swelling of nuclei and rounding of nucleoli. No increase in debris or decrease in mitoses was seen. At eleven days there appeared to be vacuolization of the reticular cells.

C. Spleen. One patient with a sarcomatous type of Hodgkin's disease was studied by means of serial splenic biopsies. The first biopsy (Fig. 9) was from tissue obtained a few hours prior to the first of two injections of 0.3 mg. per kilogram given twenty-four hours apart. The second biopsy (Fig. 10) was from tissue obtained twenty-four hours after the first injection, and the third eight days after the first injection. The edge of the spleen, which had reached to the iliac crest, receded about 5 cm. within twenty-four hours of the first injection and did not change much in size thereafter.

All three biopsies showed red pulp of the spleen in areas without any specific lymphogranulomatous lesion, although a few rare Sternberg-Reed cells were seen. The second biopsy showed distinctly fewer lymphocytes than did the first biopsy prior to treatment. The second biopsy revealed no demonstrable increase in nuclear debris, but an apparent increase in phagocytized iron. The second biopsy showed distinctly more numerous, partially collapsed, venous sinusoids per unit area than did the first biopsy, as would be expected because of the decrease in lymphocytes and shrinkage of the spleen in the interval between both biopsies. The third biopsy resembled the second but revealed more phagocytized iron and slightly smaller venous sinusoids.

II. *Lymphosarcoma*

One patient had tissue removed for biopsy by the retention-suture slicing technic, eight and one-half, seventeen, twenty-four, forty and ninety-six hours after a single dose of 0.1 mg. per kilogram (6.6 mg.). In addition to the inflammatory changes induced by this technic, it was clear that degeneration and cessation of mitoses were always much more frequent in the few tiny nodules seen than in the dense diffuse lymphatic tissue in any of the stages studied.

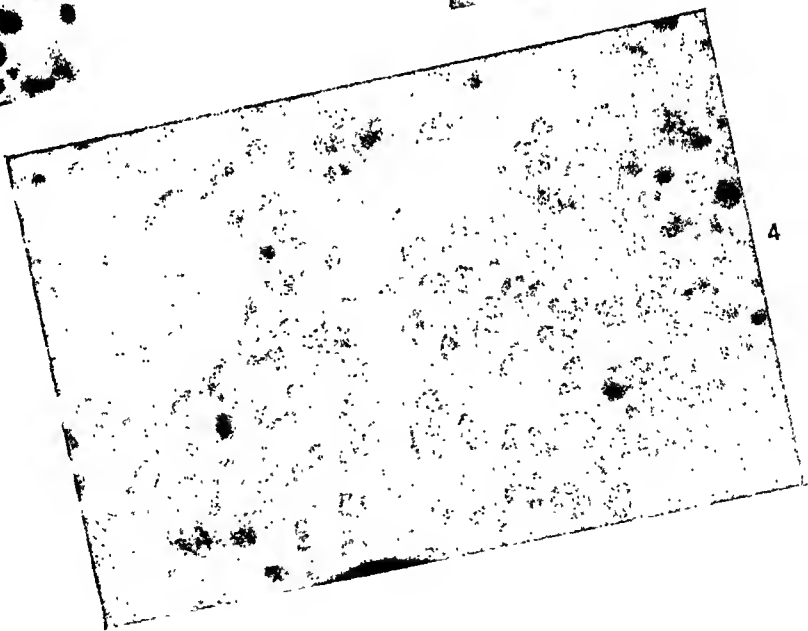
(All illustrations are from sections stained with hematoxylin eosin-azure II and photographed at a magnification of 700.)

FIG. 3. H. H., female, 31. Atrophic marrow twenty-two days after first injection. Patient was given 0.1 mg. nitrogen mustard per Kg. on each of four successive days.

FIG. 4. H. H., female, 31. Hyperplastic regenerative marrow fifty-six days after first injection. Zenker-formol osmic acid. Patient was given 0.1 mg. per Kg. on each of four successive days.

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III. Giant Follicular Lymphoblastoma

Two patients with this condition were studied. One patient was given a single dose of 0.1 mg. per kilogram. In this patient no tissue was removed immediately prior to treatment, but there were biopsies eleven months before and twelve months after treatment. A biopsy taken eleven hours after injection revealed slightly but definitely more degeneration of the small and medium lymphocytes in the inner, germinal portion of the nodule (Fig. 11) than did the other two biopsies. Degeneration in the dense diffuse internodular tissue was not increased. There was also a decrease in mitoses in the nodules. Clinically, the nodes became much smaller, but returned to their original size in about two months.

A second patient with a rather anaplastic type of giant follicular lymphoblastoma was treated with two doses of 0.2 mg. per kilogram. Tissue was obtained for biopsy by aspiration from the same node immediately prior to the first injection and again twenty-four hours later. The node, originally 7 x 3 cm. in size, decreased to one-half of its original size within twenty-four hours after the first dose. The degeneration again was in the nodules primarily, but was much more intense than that seen in the previous patient (Fig. 12). The cytotoxic changes described in these two cases were similar to those seen in the other lymph nodes. While they were qualitatively similar, they were more intense at the same dose and occurred characteristically in the nodules.

IV. Chronic Myelogenous Leukemia

One patient with a chronic myelogenous leukemia of approximately four years' duration and a profound anemia had two pretreatment splenic biopsies and biopsies at forty-four hours and ninety-six hours after the first of four daily injections of 0.1 mg. per kilogram.

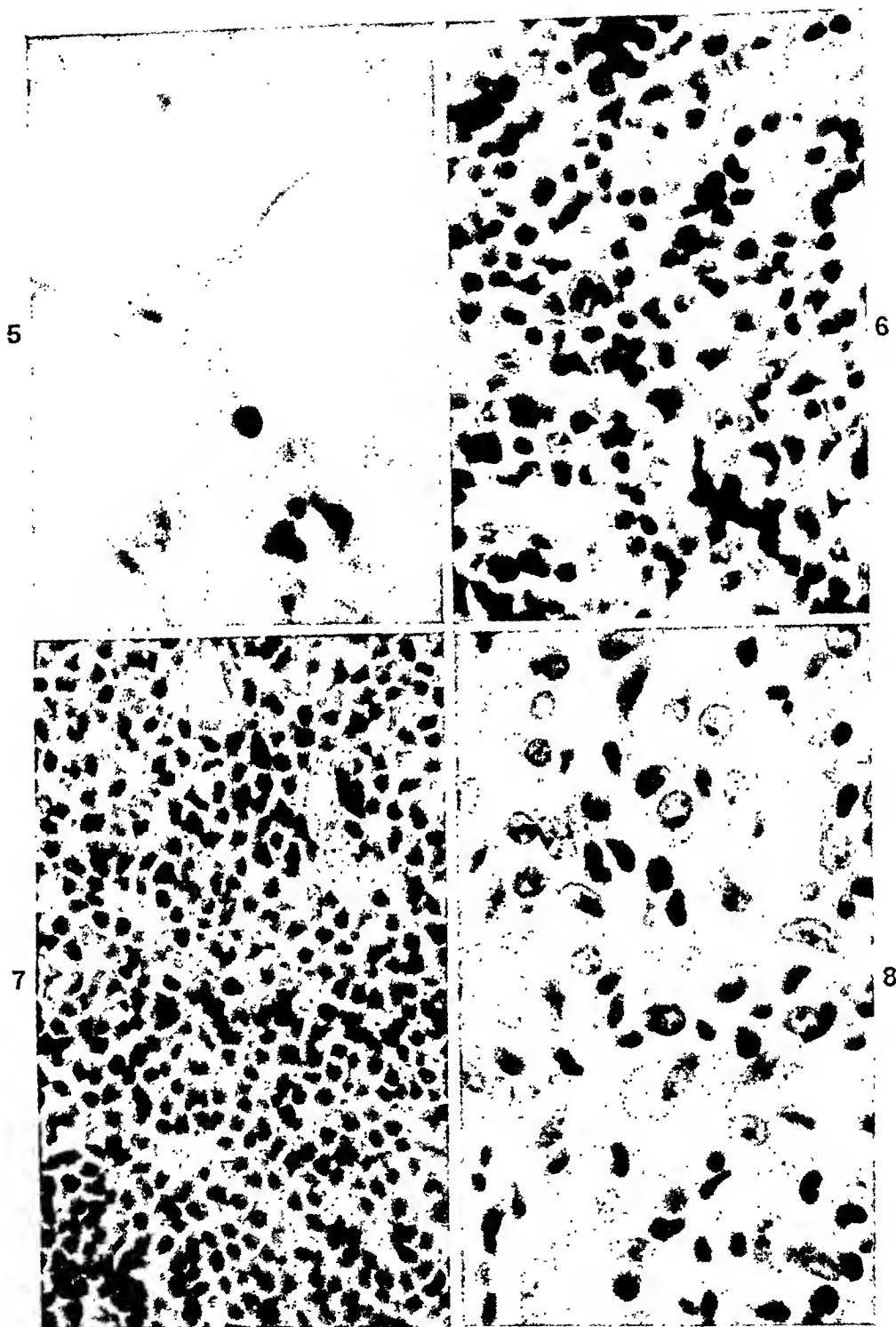
The first biopsy, obtained two months before treatment, showed significantly larger sinusoids due to engorgement by numerous erythrocytes. This was not observed in subsequent biopsies. Following treatment, the spleen became softer, but a significant histologic difference in the three biopsies was not evident. It is our impression that there were fewer myelocytes and more prominent reticular cells in the biopsies after treatment. Erythroblasts were not seen in any of the biopsies. This particular patient did not show any significant clinical improvement following therapy, and he died five months later.

V. Lymphatic Leukemia

In one patient a biopsy was obtained six days after the first of the four injections. After treatment the node had distinctly fewer lymphocytes and more prominent reticular cells perivascularly than did the node before treatment. At this time there was no significant cellular degeneration. Also, these reticular cells contained a great deal of phagocytized iron. Clinically, he had a remission lasting three months.

VI. Multiple Myeloma

One patient was studied. The biopsy, six days after the first injection, was essentially similar to that before treatment. Clinically, the patient was unchanged.



(All illustrations are from sections stained with hematoxylin eosin-azure II and photographed at a magnification of 700.)

FIG. 5. E. M., female, 32. Atrophic marrow thirty days after treatment. From patient whose stained bone marrow is illustrated in Fig. 1. Patient was given 0.1 mg. nitrogen mustard per Kg. on each of four successive days.

FIG. 6. E. M., female, 32. Lymph node thirty days after first injection

FIG. 7. J. W., male, 30. Lymph node prior to treatment.

FIG. 8. J. W., male, 30. Lymph node ten days after first injection. From patient whose lymph node before treatment is shown in Fig. 7. Patient was given 0.1 mg. per Kg. on each of four successive days.

VII. Metastatic Carcinoma

One patient with an invasive anaplastic carcinoma of unknown origin was treated with 0.1 mg. per kilogram on each of four successive days. Tissue was obtained for biopsy from a large right cervical mass prior to treatment and again two days, three days and six days after the first injection. Clinically, a transitory softening was noted at about the third or fourth injection. Histologically, no significant change could be demonstrated between the control biopsy and the biopsies after treatment.

This patient particularly illustrated the need for a control biopsy before treatment since all his biopsies revealed numerous degenerating cells and atypical mitoses.

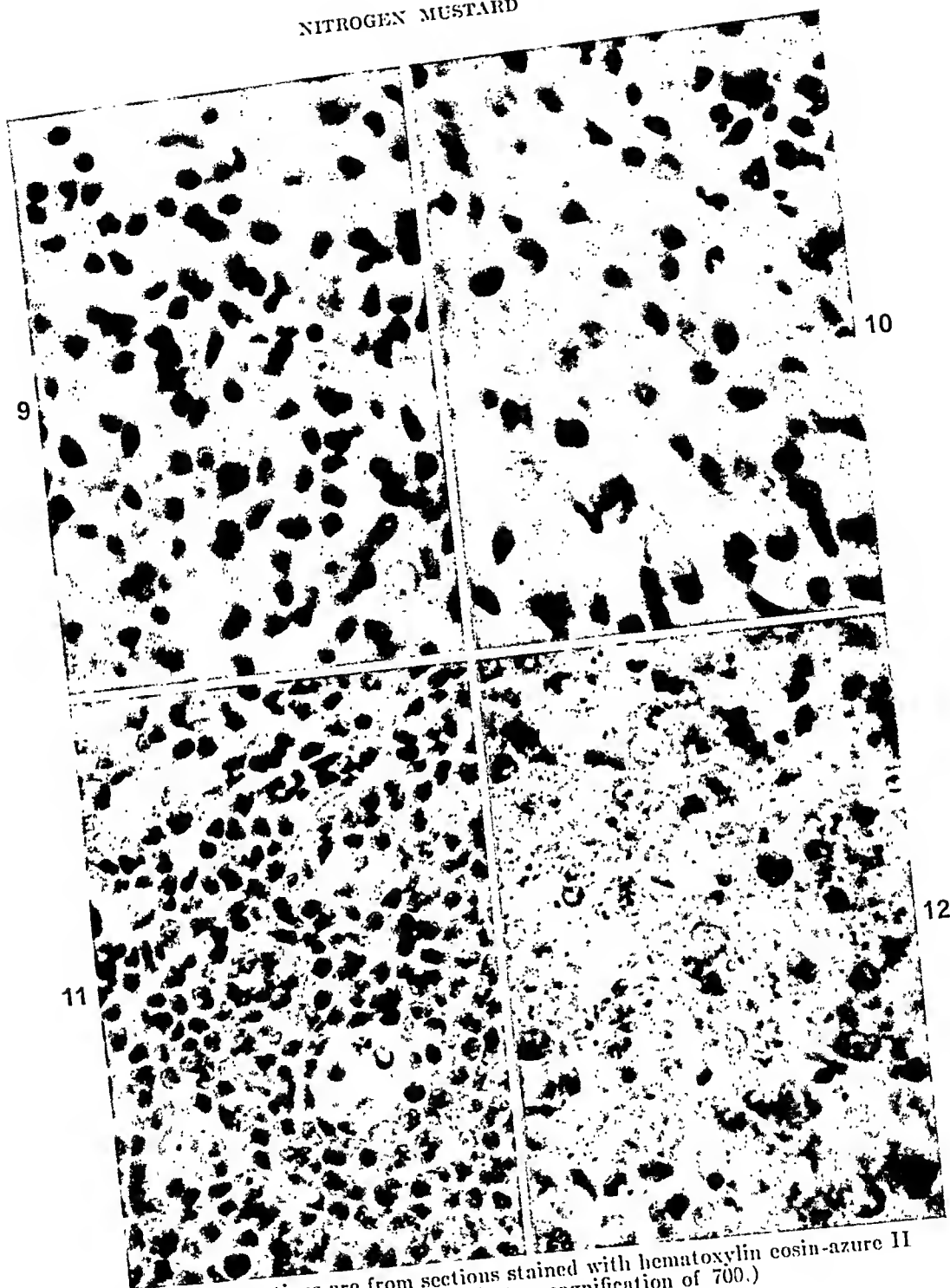
DISCUSSION

The pathologic effects of a related mustard, bis (β -chloroethyl) sulfide, were first studied during World War I by Winternitz.²⁵ However, the effect on the hematopoietic organs was not appreciated since major effort was directed to the skin and respiratory tract. In 1918 Krumbhaar and Krumbhaar¹⁴ described the changes seen at autopsy in men poisoned by mustard gas (yellow cross gas). Since all of their patients had survived at least three days, they were unaware of the acute cytotoxic changes; but they noted the atrophic and regenerative processes and the coincidental leukopenia in the peripheral blood. Also, all of their patients had a complicating severe pneumonia.

In 1920, Pappenheimer and Vance¹⁹ reported their results, in the rabbit, of injection of dichloroethylsulfide. They likewise did not study the bone marrow until four days after injection. Strangely enough, they felt that the intense necrosis of the lymphocytes seen in the first twenty-four hours (spleen and lymphatic nodules) was entirely nonspecific and not due to the mustard. However, they emphasized that the leukopenia was a result of marrow atrophy and not of destruction of the granulocytes in the peripheral blood.

Recently, Kindred¹³ has studied the acute stages of the reaction to the nitrogen mustard used in this study, the methyl-bis compound, as well as to related compounds. He has demonstrated convincingly that the small lymphocytes, erythroblasts, myelocytes and megakaryocytes are the most susceptible cells and that the reticular cells are the most resistant. Furthermore, he has clearly shown that the reduction in the number of cells in the peripheral blood is a direct result of the atrophy of the hematopoietic centers and their failure to produce cells to replace those lost from the circulating blood.

Other observations (Block²) in the rabbit have agreed essentially with those of Kindred. In brief, the following sequence of events occurs in the rabbit after the intravenous injection of a median lethal dose of nitrogen mustard. During the first eight to sixteen hours, the height of cellular destruction is attained with a cessation of mitotic activity. Beginning shortly thereafter and lasting about twelve to twenty-four hours, an active phagocytosis and digestion of nuclear debris ensues. This is followed by an atrophic period in which the reticular cells become prominent due to the disappearance of the free cells and



(All illustrations are from sections stained with hematoxylin eosin-azure II and photographed at a magnification of 700.)

- FIG. 9. V. F., female, 36. Spleen prior to treatment.
 FIG. 10. V. F., female, 36. Spleen twenty-four hours after injection of 0.3 mg. nitrogen mustard per Kg.
 FIG. 11. M. K., male, 43. Nodule from lymph node eleven hours after injection of 0.1 mg. per Kg.
 FIG. 12. G. L., male, 64. Nodule from lymph node twenty-four hours after injection of 0.3 mg. per Kg.

not because of reticular cell hyperplasia. A leukopenia coincides closely with this phase. The bone marrow is the site of a myxomatous degeneration with some erythrocytic extravasation and dilatation of venous sinusoids. At about the tenth to fourteenth day regeneration begins, homoplastically from the few susceptible cells not killed during the first twenty-four hours and heteroplastically from the reticular cells. The whole process is strikingly similar to that described by Bloom⁴ after an equivalent dose of irradiation to the entire body, except that regeneration usually is more rapid after nitrogen mustard (Block²). This is confirmatory to the sequence described from material collected by sternal aspiration following nitrogen mustard (Spurr *et al.*²²). A similar atrophic marrow may be seen after benzol injections (Pappenheimer and Vance¹⁹) and after exposure to cold wet weather (Block³).

There have been very few reports in the literature on the pathologic effects of nitrogen mustard in tumors of the hematopoietic organs. Alpert and Peterson¹ studied the histologic response of the nodes in two patients with Hodgkin's disease and described degeneration of reticular cells, eosinophils and Dorothy Reed cells. However, their illustrations appear to show very little difference between the nodes obtained before and after treatment.

Henstell, Tober and Newman⁸ have reported the effects of nitrogen mustard in 6 patients with mycosis fungoides, three of whom had biopsies before and after treatment. They stressed that the tumors with numerous "reticuloendothelial cells" are most responsive to therapy. While there is no proof that the large mononuclear cells in mycosis fungoides are "reticulo-endothelial cells", they have presented convincing evidence of the disappearance of most of the cellular infiltrate several days after treatment.

Our studies, in general, have been in agreement with the results obtained in the normal animal with regard to the susceptibility of the various cells of the hematopoietic system to nitrogen mustard. They have offered proof substantiating the experimental demonstration of the resistance of reticular cells and plasma cells to the necrotizing effects of nitrogen mustard. As would be expected, the tumors in this study that were composed primarily of reticulum cells (Hodgkin's sarcoma) and of plasma cells (multiple myeloma) were the least amenable to therapy.

Except in the nodules in some of the lymph nodes, there was little inhibition of mitoses in any cell type in contrast to the reaction seen in animals using a median lethal dose (Kindred,¹³ Block²). There are two possible explanations. First, the dose used was not large enough to duplicate the results obtained in animals. Second, in patients it is impossible to obtain enough biopsies to follow the changes as closely as may be done in the experimental animal.

The toxic reaction observed in the marrow of the patients with Hodgkin's disease, following a dose of 0.1 mg. per kilogram body weight given daily on four successive days, explains the leukopenia and recovery following therapy. The mild nature of the degeneration, the lack of any complete inhibition of mitoses and the unfailing regeneration, with occasionally a compensatory hyperactivity in the recovery stage, all indicate that this level of dosage was well within the tolerance range of all the patients studied. The sections of marrow

of patient E. M. (Figs. 1 and 5) illustrate that the marked toxic depression, even after larger doses, may still be followed by regeneration.

One would have expected a degeneration of the myelocytes in the single case of chronic myelogenous leukemia studied by means of serial splenic biopsies. However, this patient had previously been treated intensively with irradiation and urethane. His spleen was fibrous and composed of masses of reticulum cells and extremely immature promyelocytes, which do not appear to be as susceptible as the more mature myelocytes and metamyelocytes.

In no instance was the effect of the nitrogen mustard such as to restore to normal the basic architectural pattern of any of the tumor tissues. There was, therefore, no evidence that the essential nature of these diseases was altered. The more susceptible free cells were decreased in number, but the disrupted architectural pattern characteristic of involvement by malignant growth was never restored to normal.

It may be well to recall that in the normal lymph node it is impossible to dissociate functionally the reticular cells from the free cells. Embryologically, the free cells (lymphocytes) are derived from the fixed cells (cellular reticulum), and in the adult the free cells continue to be derived in part heteroplastically from the cellular reticulum as well as homoplastically from pre-existing free cells (Maximow and Bloom¹⁶). The evidence of a progression of Hodgkin's disease from a lymphocyte-rich node to one with reticulum cell hyperplasia suggests that the reticulum cells, which are extremely resistant to therapy, play a basic role in the disease.

Because of the marked similarity of the tissue reaction following roentgen irradiation and nitrogen mustard injection in the experimental animal and the similar clinical response following these two types of therapy, it was considered important to compare the pathologic changes obtained in our patients with the changes reported after irradiation therapy. Unfortunately, there is a dearth of well-controlled studies of the effects on the tissues of ionizing irradiation in these diseases, especially in the more chronic cases. Most of the reported work is based upon the findings at autopsy or in isolated biopsies at varying periods after treatment without any biopsy obtained immediately prior to treatment to serve as a control. Most of this literature has been reviewed by Prym²¹ and Lubarsch and Wätjen¹⁵ and, in their opinion, with certain rare exceptions, the changes ascribed to irradiation were all nonspecific and were characteristic of the normal progress of the disease. Our own experience in this study has amply demonstrated the necessity of controlling all observations by biopsies taken immediately prior to therapy, and we have rejected numerous biopsies from this study for this reason.

Heineke⁷ in a case of bilateral mammary lymphosarcoma noted that the right breast, five hours after irradiation, had numerous nuclear fragments and splotchy chromatin particles in many of the residual nuclei, as compared to the left (unirradiated) breast. He concluded that the histologic picture after irradiation of this sarcoma was identical with what he had seen under similar circumstances in normal irradiated animals.

The acute cytotoxic stage following irradiation has been studied in a lymph

node of a patient with lymphatic leukemia by Houdé⁹ and later by Ménétrier and Touraine.¹⁷ In well-controlled material obtained three days after irradiation, they have illustrated a decrease in lymphocytes, degeneration of small lymphocytes and phagocytosis of nuclear fragments by macrophages. The larger lymphocytes and "endothelial cells" were comparatively undamaged.

Weil and Perles²⁴ reported chronic myelogenous leukemia in a patient from whom repeated splenic, liver and marrow biopsies were taken over the course of the disease, about fifteen months. After the first irradiation there was a reversion towards the normal cellular distribution, as seen in dry smears, so that it was impossible to make a diagnosis of myelogenous leukemia from the smears of the marrow although those from liver and spleen were suggestive of myelogenous leukemia. However, during the succeeding few months of the patient's life, when the patient's smears had more immature cells, there was no clinical or cytologic response to therapy. Unfortunately, only smears were made, and no conclusions can be drawn about effects on the basic architecture after therapy.

Stephen,²³ in a report of chronic myelogenous leukemia of four years' duration, described the change from a marrow characteristic of myelogenous leukemia to a marrow composed of loose connective tissue with occasional myelocytic and erythropoietic islands. The biopsy was taken about two or three weeks after therapy, at the height of clinical remission. It resembles qualitatively the marrow seen at a comparable time after a median lethal dose of nitrogen mustard in rabbits and in our patient, E. M., although there was not as marked a decrease in hematopoiesis in Stephen's case. Interestingly, he described a similar change after Fowler's solution in another patient with myelogenous leukemia. Stephen emphasized that the marrows did not return to a normal architecture in spite of the profound changes induced by treatment.

Moeschlin¹⁸ studied two patients with chronic myelogenous leukemia by means of serial splenic biopsies obtained prior to and following splenic irradiation. In the first patient at twenty-four hours after exposure to 120 r, he noted a myelocytic degeneration, decrease in immature cells and an increase in erythroblasts. In the second patient, four months after a course of treatment totalling 800 r, there was a decrease in myelocytes and to a lesser degree, in erythroblasts. There was also an increase in mature granulocytes. He concluded that irradiation destroys the myelocytes, inhibits mitoses and increases the percentage of mature neutrophils. As in the patient of Weil and Perles,²⁴ no sections were made and so no statement can be made about the architectural pattern of the tissues.

Pautrier²⁹ illustrated the effect of irradiation on the cutaneous tumors of mycosis fungoides by comparing a biopsy obtained prior to treatment with those obtained four and seven days afterwards. His results were strikingly similar to those reported by Henstell *et al.*⁸

Brunschwig and Kandel⁵ described the changes in nodes taken from patients with Hodgkin's disease and lymphosarcoma following irradiation. They noted a decrease in lymphocytes and increase in reticular cells and sclerosis in Hodgkin's

disease after irradiation. However, biopsies were not obtained prior to each course of therapy, and it is difficult to be sure that the changes were all due to the therapy and were not a spontaneous result of the progress of the disease. In lymphosarcoma in two patients with a more malignant course, there was some slight sclerosis after treatment, but no change was seen in the less malignant cases. In their report of these two diseases no mention was made of reversion to normal architecture following treatment nor was any effect of treatment upon the reticular cells described.

In summary, it would appear that the pathologic effects of both irradiation and nitrogen mustard are at least qualitatively similar in that similar cell types undergo necrosis in both the experimental animal and in patients with tumors of the blood-forming organs. The small lymphocyte, the myelocyte and erythroblast are the most susceptible cells after both types of therapy. On the other hand, the reticular cells and plasma cells are extremely resistant. There is also striking similarity in the failure of both therapeutic agents to induce a return to a normal architecture. In Hodgkin's disease after irradiation, as after nitrogen mustard therapy, there is described the usual decrease in lymphocytes and tendency to sclerosis. Although this may coincide with a clinical remission, it is well to remember that the reticular cells still may retain their ability to regenerate the cells destroyed by treatment. Even if this does not occur, the disease is fatal in a comparatively short time since the reticular cells are presumably part of the malignant process.

CONCLUSIONS

A histologic study was made of lymph nodes, bone marrow and spleen in patients with neoplastic disease of the hematopoietic tissues before and after nitrogen mustard therapy. On the basis of these studies the following conclusions have been made:

1. The bone marrow exhibits a very slight increase in degeneration within twenty-four hours after an injection of nitrogen mustard. This is followed by an atrophic phase, resulting in a peripheral leukopenia with a less intense anemia and thrombocytopenia. After a therapeutic dose, regeneration always occurs and hyperplasia is often found within thirty days after the first injection.
2. Although certain slight changes in the histologic appearance of the nodes in Hodgkin's disease of the paraganulomatous-granulomatous type are probably demonstrable, they are not commensurate with the decrease in the size of the nodes seen clinically. However, it is possible that more striking changes would be seen if nodes were examined at biopsy at additional intervals after treatment.
3. The lymph nodes in Hodgkin's sarcoma show very little, if any, histologic change following therapy. This correlates with a comparatively short clinical remission.
4. There is always regeneration of the bone marrow and lymph nodes after administration of nitrogen mustard in doses as high as 60 mg. given in a single course within seven days.
5. In lymphosarcoma and lymphatic leukemia there is evidence of a fairly

intense lymphocytic destruction, always more marked in the nodules. A similar effect, restricted to perivenous tissue, is apparent in lymphatic leukemia.

6. In a miscellaneous group (multiple myeloma, myelogenous leukemia and metastatic carcinoma) of far-advanced malignant neoplasms, limited observations have failed to demonstrate any significant clinical or histologic change following nitrogen mustard therapy.

7. It is probable that nitrogen mustard exerts its principal cytotoxic effect on the smaller lymphocytes, myelocytes, erythroblasts, and megakaryocytes. The reticular and plasma cells are the most resistant hematopoietic cells.

8. Comparative histologic observations on the effects of nitrogen mustards and ionizing radiations on the hematopoietic tissue of experimental animals and human subjects with neoplasms of these tissues indicate that after most of the more susceptible cells are destroyed, regeneration inevitably results homoplastically from the few residual susceptible cells and heteroplastically from the reticular cells.

9. It is probable that methods of therapy of neoplastic diseases of the hematopoietic tissues, which rely upon a destruction of the more susceptible cells, are doomed to failure because of the reserve of immature reticular cells which serve as a source of regeneration of the other cells and carry on the malignant properties of the disease.

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INTERPRETATION OF Rh ANTIBODIES*

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This paper deals with the interpretation of Rh antibodies in maternal serum and is based on a study of the antibodies in 182 mothers of erythroblastotic babies.

The main criteria for the evaluation of serologic tests for antibodies are (1) specificity and (2) sensitiveness. As a rule, the two qualities do not run parallel in the case of an individual antibody. For example, in tests for syphilis, false negative tests (low sensitiveness) and false positive tests (low specificity) present a serious diagnostic problem. In infectious mononucleosis, the differential test^{1, 3} has thus far been found 100 per cent specific, but its sensitiveness varies from 60 to 80 per cent, depending on the indications employed for the clinical diagnosis of the disease. On the other hand, tests for Rh antibodies occupy the unique position of having a very high degree (over 95 per cent) of sensitiveness and a specificity of 100 per cent. Thus far, not a single case has been reported in which Rh antibodies were found without previous introduction of the Rh antigen. The introduction of human plasma or serum and of bovine albumin as diluents has raised the sensitiveness of tests for Rh antibodies to this very high level.

The discovery of four different tests for Rh antibodies suggested the possibility that there may be a relationship between them and the different forms of fetal erythroblastosis. The four techniques are: 1. tests in which saline is used as diluent: tests for saline agglutinins, Wiener's bivalent agglutinins, Diamond's thermolabile agglutinins; 2. tests in which human plasma or serum or bovine albumin is used as diluent: tests for serum albumin agglutinins, Wiener's conglutinins, glutinins, or univalent agglutinins, Diamond's thermostabile agglutinins; 3. Wiener's test for blocking antibodies (incomplete antibodies of Race and Taylor); 4. Coombs' test (Hill's developing test).

Do these tests show only varying degrees of immunization or are the differences qualitative in nature? Is it possible to predict the outcome of pregnancy from the study of Rh antibodies during pregnancy?

A few examples may be given.

In case 419 (Table 1), the mother had an unfavorable obstetrical history. Serum albumin agglutinins were present on the first examination in the twelfth week of pregnancy. In view of the past history, Rh antibodies found during the first trimester must be interpreted as carried over from previous sensitization. There was a moderate rise in the later course and, in addition, blocking antibodies appeared. The outcome was a stillborn hydropic infant with typical fetal erythroblastosis confirmed by necropsy.

* Aided by a grant from the Committee on Scientific Research of the American Medical Association. Presented at the Twenty-Sixth Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 29, 1947. Received for publication, May 3, 1948.

In the second case (No. 842, Table 1), the obstetrical history was also unfavorable. Antibodies were present in the mother's serum in the eighth week of the fifth pregnancy, and in a much higher titer after spontaneous abortion four weeks later. When the mother returned ten months later she was six weeks pregnant and the titer was again very high. What prognosis could be given in this case? Two circumstances permitted some hope: 1. The father was heterozygous (he was Rh-positive, Hr-positive, and the first child was Rh-negative). There was a possibility of an Rh-negative fetus. 2. The antibodies, at least those at beginning of the pregnancy, were probably of the "carry-over" type. What degree of reliance may be placed upon such indicators? What is the significance of a fall in the titer towards the end of the pregnancy? In this instance, an Rh-negative normal infant was born at term.

Does the outcome of the pregnancy permit conclusions that may be applied to other cases, for instance to Case 866 (Table 2)? Here antibodies were present in the eighth week of pregnancy. The possibility that the antibodies were of the "carry-over" variety was the only hope that could be held out in this case. The fetus had to be Rh-positive in view of the father's homozygosity. Could any significance be attached to the fact that the antibodies remained at a low level through the entire pregnancy? . . . The baby that was born had a moderately severe anemic form of erythroblastosis, but recovered after several transfusions of Rh-negative blood.

In Case 1373 (Table 2) the situation, as reflected by the level of antibodies, differed from the preceding only in the heterozygosity of the father, offering hope that the infant could be Rh-negative. . . . An Rh-negative healthy baby was born.

In the next case (No. 386/47, Table 2), when the mother was first examined, the pregnancy was far advanced. The obstetrical history was normal, and the study of the antibodies gave findings which are listed in the table. There was good reason to anticipate disease in the infant, but the outcome was a pleasant surprise! Many similar examples could be enumerated without finding a sure formula to prognostication in any individual case.

Wiener was the first to attempt a correlation between the various forms of Rh antibodies in pregnant women and the clinical manifestations in the erythroblastotic infants. He stated that "bivalent" agglutinins (for which the senior author has suggested the term, saline agglutinins) are mainly responsible for icterus gravis, and that the so-called conglutinins (serum albumin agglutinins) and blockers (blocking antibodies) or "univalent" antibodies are responsible for the severe damage leading to hydrops and intrauterine death.⁵ In his series, agglutinins alone (saline agglutinins) were found in 64 per cent of mothers whose babies had icterus gravis, whereas blockers and conglutinins were present in only 14 per cent of such mothers. Only 8 per cent of mothers of still-born babies were found to have saline agglutinins whereas 43.9 per cent had conglutinins and blockers.

One of us² reported a similar study in 73 mothers of babies with erythroblastosis at the Dallas Rh conference in November 1946. The results were

TABLE 1

CASE 419. OBSTETRICAL HISTORY AND RH ANTIBODIES IN MOTHER. FATHER GROUP A, RH-POSITIVE; MOTHER GROUP A, RH-NEGATIVE

PREGNANCIES			TITER OF ANTIBODIES		
Date	No.	Outcome	Saline Agglutinins	Serum Albumin Agglutinins	Blocking Antibodies
1932	1	Normal child			
1934	2	Induced abortion			
1937	3	Induced abortion			
1939	4	Stillbirth at term			
1942	5	Stillbirth, premature			
1944	6	Stillbirth, fetal erythroblastosis at term			
Nov. 6, 1946 (Pregnant 12 wk.)	7		0	1:5	0
Jan. 27, 1947			0	1:10	1:5
March 3, 1947			0	1:40	1:10
April 3, 1947			0	1:10	1:1
April 18, 1947			0	1:80	1:1
April 21, 1947			0	1:40	1:1
May 3, 1947		Stillbirth, fetal erythroblastosis and hydrops			

CASE 842. FATHER GROUP O, RH-POSITIVE, HR-POSITIVE; MOTHER GROUP O, RH-NEGATIVE

PREGNANCIES			TITER OF ANTIBODIES		
Date	No.	Outcome	Saline Agglutinins	Serum Albumin Agglutinins	Blocking Antibodies
1935	1	Normal child, O, Rh-neg.			
1938	2	Normal child, O, Rh-pos.			
1944	3	Stillbirth, 6 months			
1945	4	Stillbirth at term, fetal erythroblastosis and hydrops			
Dec. 7, 1945 (Pregnant 8 wk.)	5		0	1:640	1:80
Jan. 10, 1946		Spontaneous abortion, 12 weeks	0	1:1280	1:640
Oct. 1, 1946 (Pregnant 6 wk.)	6		0	1:2560	1:320
Jan. 20, 1947			0	1:5120	1:20
March 7, 1947			0	1:320	1:10
April 22, 1947			0	1:320	1:10
April 30, 1947		Normal infant born, O, Rh-negative			

similar to those of Wiener. Since the Dallas meeting, 109 additional cases have been analyzed. The present report is based on a study of the antibodies in 182 mothers of erythroblastotic babies.

TABLE 2

CASE 866. FATHER GROUP A, RH-POSITIVE, HR-NEGATIVE; MOTHER GROUP O, RH-NEGATIVE

PREGNANCIES			TITER OF ANTIBODIES		
Date	No.	Outcome	Saline Agglutinins	Serum Albumin Agglutinins	Blocking Antibodies
1938	1	O, Rh-positive, normal			
1940	2	O, Rh-positive, normal			
1945	3	O, Rh-positive, kernicterus			
August 22, 1946 (Pregnant 8 wk.)	4		0	1:20	1:5
Oct. 4, 1946			0	1:20	1:10
Nov. 11, 1946			0	1:10	1:5
Jan. 15, 1947			0	1:10	1:1
March 10, 1947		O, Rh-positive infant born; fetal erythroblastosis, anemia; survived			

CASE 1373. FATHER GROUP O, RH-POSITIVE, HR-POSITIVE; MOTHER GROUP A, RH-NEGATIVE

PREGNANCIES			TITER OF ANTIBODIES		
Date	No.	Outcome	Saline Agglutinins	Serum Albumin Agglutinins	Blocking Antibodies
1943	1	A, Rh-positive, normal			
Jan. 1946	2	Icterus gravis; died			
Sept. 18, 1946			0	1:10	1:1
March 27, 1947 (Pregnant 12 wk.)	3		0	1:80	1:1
April 29, 1947			0	1:80	1:1
June 18, 1947			0	1:40	1:1
July 21, 1947			0	1:40	1:20
Aug. 30, 1947			0	1:40	1:20
Oct. 30, 1947		Normal infant born, A, Rh-negative			

CASE 386/47. FATHER GROUP B, RH-POSITIVE; MOTHER GROUP O, RH-NEGATIVE

PREGNANCIES			TITER OF ANTIBODIES		
Date	No.	Outcome	Saline Agglutinins	Serum Albumin Agglutinins	Blocking Antibodies
1939	1	O, Rh-positive, normal			
1941	2	B, Rh-positive, normal			
June 13, 1947 (Pregnant 7½ mo.)	3		1:1	1:10	0
June 24, 1947			1:1	1:20	0
July 8, 1947			1:5	1:10	0
July 21, 1947			1:5	1:40	0
July 26, 1947		O, Rh-positive, normal infant born			

TABLE 3

RELATIONSHIP BETWEEN PRESENCE OF VARIOUS RH ANTIBODIES IN MATERNAL SERUM AND
OUTCOME OF PREGNANCY
TYPE AND PRESENCE OF ANTIBODIES

	NO.	BLOCKING ANTIBODIES: PRESENT SERUM ALBUMIN AGGLUTININS: PRESENT SALINE AGGLUTININS: ABSENT			BLOCKING ANTI- BODIES: ABSENT SERUM ALBUMIN AG- GLUTININS: PRESENT SALINE AG- GLUTININS: PRESENT	
		No.	Per Cent	P.E.*	No.	Per Cent
Total births	182	73	40.1	2.5	109	59.9
Deaths	103	49	47.6	3.3	54	52.4
Stillbirths and hydrops	54	37	68.5	4.3	17	31.5
Other deaths	49	12	24.5	4.2	37	75.5
Survivals	79	24	30.4	3.5	55	69.6
Icterus gravis	107	25	23.4	2.8	82	76.6
Mothers who received transfusion prior to birth of affected child	35	29	82.9	4.3	6†	17.1

* P.E. is the probable error of the given percentage, plus or minus. The values of the probable errors in this and subsequent tables were calculated from the formula:

$$P.E. = \pm 0.675 \sqrt{\frac{pq}{n}}$$

where p is the given percentage, q is 100 minus p , and n is the total number of cases subjected to analysis.

† Of these six mothers, two had stillborn babies, and one a baby with hydrops.

TABLE 4

CORRELATION BETWEEN PRESENCE OF VARIOUS RH ANTIBODIES IN MATERNAL SERUM AND
CLINICAL MANIFESTATIONS IN INFANT OR FETUS

TYPE AND PRESENCE OF ANTIBODIES	NO. OF FAMILIES	STILLBIRTHS AND HYDROPS			ICTERUS GRAVIS			HEMOLYTIC ANEMIA		
		No.	Per Cent	P.E.	No.	Per Cent	P.E.	No.	Per Cent	P.E.
Blocking antibodies: present Serum albumin agglutinins: present Saline agglutinins: absent	73	37	50.7	4.0	25	34.2	3.8	11	15.1	2.8
Blocking antibodies: absent Saline agglutinins: present Serum albumin agglutinins: present	109	17	15.6	2.3	82	75.2	2.8	10	9.2	1.9
Differences in percentages \pm P.E.		35.1 \pm 4.6			41.0 \pm 4.7			5.9 \pm 3.4		

Table 3 presents a statistical analysis, according to presence or absence of blocking antibodies, in the mothers of the entire series and of groups of mothers arranged according to the outcome of pregnancy.

In the total of 182 cases forming this series, 40.1 per cent had blocking antibodies in the maternal serum, while 59.9 per cent did not have them. This incidence may depend on the chance selection of the cases making up this series. However, the figures can be used as a basis of comparison for the findings obtained in the further analysis. In a group which includes mothers of all deceased infants, the incidence of blocking antibodies does not differ significantly from the incidence in the whole series.

When the deaths were divided into two groups, (a) those due to stillbirth or hydrops and (b) those due to other causes, a significant difference appears: in

TABLE 5

RELATIONSHIP OF TITER OF SERUM ALBUMIN AGGLUTININS AND PRESENCE OF BLOCKING ANTIBODIES IN MATERNAL SERUM BEFORE DELIVERY TO SURVIVAL OF INFANTS

TITER OF SERUM ALBUMIN AGGLUTININS	TOTAL CASES	DEATH OF INFANTS			SURVIVAL OF INFANTS	
		No.	Per Cent	P.E.	No.	Per Cent
1:10 or less	25	8	32.0	6.3	17	68.0
(a) blocking antibodies: absent	18	5	27.8	7.2	13	72.2
(b) blocking antibodies: present	7	3	42.9	12.6	4	57.1
Above 1:10	49	32	65.3	4.6	17	34.7
(a) blocking antibodies: absent	28	17	60.7	6.2	11	39.3
(b) blocking antibodies: present	21	15	71.4	6.7	6	28.6

mothers of the first group, the incidence of blocking antibodies is increased as compared with the incidence of these antibodies in the whole series, the difference in percentages, 28.4, being more than 5 times the probable error of ± 5.2 .* In mothers of the second group, saline agglutinins predominated as compared with the entire series, the difference in percentages, 15.6, being more than three times the probable error of ± 4.9 .

In mothers of infants that survived, saline agglutinins were somewhat more frequent than in the whole series, the difference in percentages, 9.7, being more than twice the probable error of ± 4.3 .

In the presence of icterus gravis, saline agglutinins occurred in more than three-fourths of the mothers, and the difference of percentages, 16.7, was greater than 4 times the probable error of ± 3.8 . Finally, the most significant difference was exhibited in the group of mothers who had received transfusions prior

* The probable error of the difference (in this case, difference between 68.5 and 40.1 per cent) was calculated from the formula: $P.E._{diff} = \sqrt{P.E._1^2 + P.E._2^2}$ where $P.E._1$ and $P.E._2$ are the corresponding probable errors of the compared percentages, as listed in the tables.

to birth of the affected child. More than four-fifths of this group had blocking antibodies, and the difference in percentages, 42.8, was more than 8 times as great as the probable error of ± 5.2 .

The arrangement in Table 4 is of greater immediate interest because from it one may try to answer the following question which is uppermost in the mind of the clinical pathologist: What conclusions may be drawn from the study of blood specimens obtained during pregnancy relative to the outcome of the pregnancy?

The material was divided into two groups:

In 73 mothers blocking antibodies were present; in 109 mothers they were absent. Each of these groups of mothers was then subdivided into three classes

TABLE 6

RELATIONSHIP OF PRESENCE OF ANTIBODIES IN MATERNAL SERUM AND PREVIOUS ADMINISTRATION OF TRANSFUSION TO MOTHERS, TO SURVIVAL OF INFANTS

	Grouping of Mothers According to History of Transfusion	Total Cases	Death of Infants			Survival of Infants	
			No.	Per Cent	P.E.	No	Per Cent
I. Blocking antibodies: present	(a) No transfusion	44	32	72.7	4.5	12	27.3
Serum albumin agglutinins: present	(b) History of transfusion	29	17	58.6	6.2	12	41.4
Saline agglutinins: absent	(c) (a) plus (b)	73	49	67.1	3.7	24	32.9
II. Blocking antibodies: absent	(a) No transfusion	103	51	49.5	3.3	52	50.5
Saline agglutinins: present	(b) History of transfusion	6	3	50.0	20.4	3	50.0
Serum albumin agglutinins: present	(c) (a) plus (b)	109	54	49.5	3.2	55	50.5

according to the clinical condition of the infant: stillborn infants or infants with hydrops, infants with icterus gravis, and infants with hemolytic anemia. The last-mentioned group was small and included only cases where the hemolytic anemia was the predominant finding and where jaundice was either absent or insignificant. In the presence of blocking antibodies in the maternal serum, the chances for birth of an infant that is stillborn or has hydrops are about even while the chance for an infant with icterus gravis is about 1 in 3. In the absence of blocking antibodies in the maternal serum, the chance for the occurrence of stillbirth or hydrops is about 1 in 6, while 75 per cent of the infants developed icterus gravis.

The difference in occurrence of stillborn or hydropic infants in mothers with blocking antibodies as compared with those without blocking antibodies is significant, being more than 7 times the probable error. The difference in occurrence of icterus gravis in mothers without blocking antibodies as compared

with those with blocking antibodies, is also highly significant, being more than 8 times the probable error. The per cent incidence of hemolytic anemia is slightly higher in the group with blocking antibodies, but the difference is not significant.

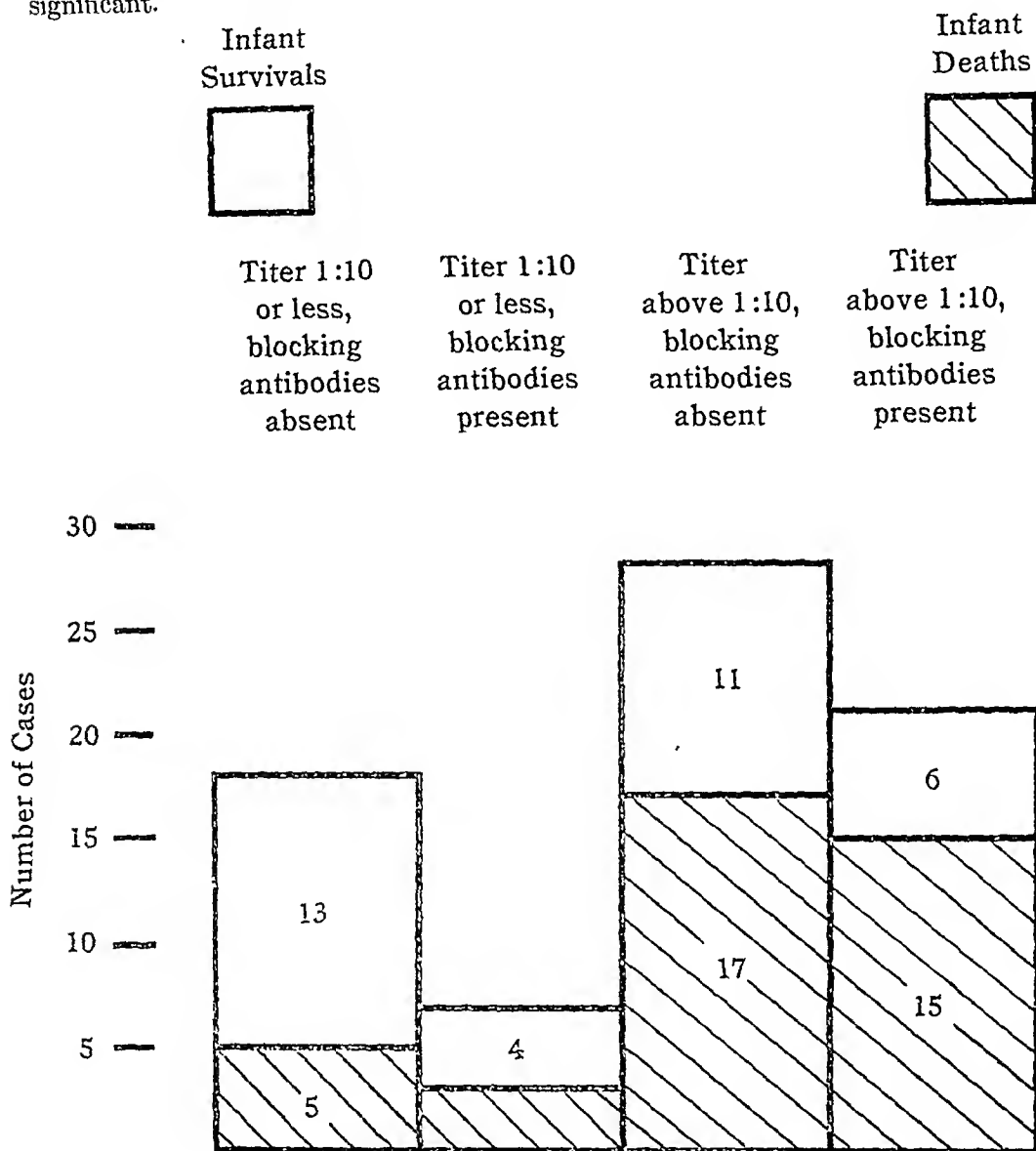


FIG. 1

Relation of Titer of Serum Albumin Agglutinins in Maternal Serum Before Delivery to Survival of Infants

Sacks and associates¹ found a significantly higher mortality in erythroblastotic infants when the titer of serum albumin agglutinins in the mothers was higher than 1:10. Table 5 and Figure 1 are concerned with 74 cases of our series in which antibodies could be studied before delivery.

The 74 cases were subdivided into two groups according to the highest titers of serum albumin agglutinins. The first group includes cases with a titer of 1:10 or less, the second those with a titer higher than 1:10. In each group the death or survival of the infant is recorded. In the first group about one-third of the children died and two-thirds survived, while in the second group, the proportions of deaths and survivals were reversed. The difference between the death and survival rates in the two groups, 33.3 per cent, is statistically significant, being more than 4 times the probable error of ± 7.8 .

The presence of blocking antibodies adds an additional aggravating factor which may be lethal, as evidenced by the fact that the mortality was 71.4 per cent in the cases with a titer above 1:10 and with blocking antibodies, 42.9 per cent with blocking antibodies and a titer less than 1:10, 60.7 per cent with a titer above 1:10 without blocking antibodies, and only 27.8 per cent with a titer of 1:10 or less without blocking antibodies.

When the difference in the death or survival rate between the subgroups, (a) or (b), is calculated, it is found statistically significant: the difference of 32.9 per cent between the death rate in the two subgroups without blocking antibodies is more than 3 times as great as the probable error (± 9.5). The difference of 28.5 per cent between the subgroups with blocking antibodies is just at the limit of significance, being about twice the probable error of 14.3. Here the smallness of the sample depresses the statistical significance. The above figures appear to indicate that two factors, *viz.*, high antibody titers and presence of blocking antibodies, occurring singly or together, lower the chance of the survival of the infant. The death rate was found highest when both factors were present, and the survival rate was highest when both factors were absent.

In view of the high incidence of blocking antibodies in women who had received transfusions prior to the birth of erythroblastotic children, the effect of transfusions on the survival of infants was studied (Table 6). The entire series of 182 cases was again divided into two main groups: one in which blocking antibodies were present in the maternal serum, the other in which no blocking antibodies were present. In both groups the numbers and percentages of death and survival of infants were charted. In the presence of blocking antibodies only about one-third of the infants survived, (32.9 per cent), while in the absence of blocking antibodies about one-half survived (50.5 per cent). The difference between these values (17.6 per cent) is more than 3 times the probable error of ± 4.9 .

In both main groups, subgroups were created by separating mothers who had received transfusions from those who did not. In the group without blocking antibodies, previous transfusion to the mother failed to change the death and survival rates of their infants. However, in the presence of blocking antibodies in the maternal serum, there is a higher death rate and a lower survival rate of infants born to mothers who did not receive transfusions, than in infants whose mothers had received transfusion, but the difference in percentage between these two subgroups is not significant.

As mentioned previously, the difference in percentages of deaths and survivals of the two main groups is significant. If the subgroup of mothers having blocking antibodies who had no previous transfusion (Ia) is compared with the group of mothers without blocking antibodies who had no previous transfusion (IIa), the difference between the death and survival rates of their infants is found to be 23.2 per cent, which is statistically significant, being about 4 times the probable error of ± 5.6 . However, comparison of the two subgroups of mothers who had received transfusion (Ib and IIb) reveals a difference of only 8.6, which is statistically insignificant (probable error ± 21.3). The sampling of mothers who had received transfusions and failed to show blocking antibodies in their serum, is very small, owing to the fact that a previous transfusion so frequently gives rise to blocking antibodies. It is possible that the significance of blocking antibodies is different in women with a history of transfusion than in those without such a history. In women with a history of transfusion, the presence of blocking antibodies appears in our material to be less ominous for the fate of the child, than in those having blocking antibodies but no history of a transfusion. It should be emphasized that the differences are at the border of statistical significance.

CONCLUSIONS

1. The prognosis of the outcome of pregnancy and the chances of survival of the infant are made less favorable by the finding of blocking antibodies, except when there is a history of a blood transfusion, or a possibility that such antibodies may have been carried over from a previous pregnancy.

2. Heterozygosity of the father with regard to the Rh factor permits one to hold out the consoling possibility that the mother may give birth to a healthy Rh-negative baby, even though she has had an unfavorable obstetrical history and Rh antibodies in her serum during pregnancy, provided that the latter were found during the first trimester.

3. Elevated titers of Rh antibodies (serum albumin Rh antibodies) in the mother reduce the chances of survival of the newborn.

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MASSIVE NECROSIS OF LIVER FOLLOWING EXCHANGE TRANSFUSION FOR ERYTHROBLASTOSIS FETALIS*

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In exchange or substitution transfusion for erythroblastosis fetalis, methods which differ slightly in technic and in the blood vessel used, have been developed by Polayes,¹⁴ Wiener and Wexler²⁴ and by Wallerstein.²¹ The blood of an erythroblastotic infant is drained and simultaneously replaced by citrated compatible donor's blood. The total amount of blood used varies from 500 to 1000 cc. (1 to 2 units). By means of this procedure, and sometimes by the additional help of heparin as a systemic anticoagulant, they have so speeded the operation that a 90 per cent exchange of blood may be effected in about ninety minutes.

It is the purpose of this communication to report the necropsy findings in three infants who died following such exchange transfusions. The lesions were strikingly different from those ordinarily seen in erythroblastosis fetalis.

CASE 1†

Clinical Data

J. L., a white male infant, was born November 1, 1946 in another hospital and died five days later. The mother was a gravida V, para II. The third and fourth pregnancies terminated in the birth of stillborn premature infants. In August 1946, while in her fifth month of pregnancy, the mother and the members of her family were examined to determine their blood groups and Rh-Hr factors. The results were as follows:

	Group	M-N Type	Rh-Hr Type
Father	O	MN	Rh ₁ Rh ₁
Mother	O	M	rh
First son	O	M	Rh ₁ rh
Second son	O	MN	Rh ₁ rh

Thus, the mother was Rh-negative and her husband homozygously Rh-positive. The expected infant had to be group O, type Rh-positive (Rh₁rh). Tests for Rh antibodies in the mother's serum revealed the following: agglutinating antibodies, 4 units (weak reaction); conglutination test, 4 units (strong reaction). This proved that the mother was sensitized to the Rh factor and that there was a mixture of univalent and bivalent antibodies in her blood serum.

Because of these findings, the infant was delivered by cesarean section six weeks before term. The cord was bile stained, and the infant appeared somewhat pale at birth. Serologic study of the infant's blood showed it to belong to group O, type MN and to have the Rh₁rh factor. The hemoglobin was 80 per cent (Haden-Hausser), the red blood cell count 2,620,000 per cu. mm., and the leukocyte count 23,200 per cu. mm. with 50 per cent polymorphonuclear leukocytes, 43 per cent lymphocytes, 4 per cent monocytes, and 3 per cent

* Received for publication, April 29, 1948.

† I am indebted to Dr. A. S. Wiener for the serologic data in these cases.

eosinophils. There were 18 nucleated erythrocytes per 100 white blood cells. The icterus index at birth was 70.

An immediate exchange transfusion was carried out using 500 cc. of group O Rh-negative blood to which had been added 60 cc. of 2.9 per cent sodium citrate solution in normal saline. Three doses of 0.2 cc. of heparin were injected into the saphenous vein during the procedure. A total of 420 cc. of blood was withdrawn while 500 cc. was injected. The procedure took about ninety minutes. It was not stated whether calcium gluconate was given.

The next day the infant seemed to be doing well, but jaundice was deeper. The hemoglobin was 135 per cent and the red blood cell count was 6,700,000 per cu. mm. Differential agglutination showed that a 90 per cent replacement of the infant's blood had been accomplished. On the third day of life the icterus index was 110. The baby took its feedings poorly. Brawny edema of the legs became apparent, but the spleen and liver were not palpable. At this time the hemoglobin was 100 per cent and the erythrocyte count 5,200,000 per cu. mm. On the fourth day of life the infant appeared dehydrated and was transferred to the Jewish Hospital of Brooklyn. Physical examination revealed a deeply jaundiced infant whose cry was of "cerebral type". The liver and spleen were not palpable. Glucose and saline, and glucose in distilled water were administered parenterally. He was lethargic and took his feedings poorly. During one of these feedings, the infant aspirated part of the formula, an attack of cyanosis ensued and shortly thereafter he expired.

Necropsy Findings

(Only the pertinent findings will be given in this and in the subsequent cases.)

Gross description. The body was that of a well developed, well nourished, white male infant, measuring 50 cm. in length and weighing 3200 Gm. The sclerae, skin and mucous membranes were icteric. There was moderate pretibial edema. There was no excess of fluid in the body cavities. The *thymus* weighed 4 Gm. and revealed a few subcapsular hemorrhagic areas which measured up to 3 mm. in diameter. The *lungs* were crepitant, and the cut surfaces showed a few small hemorrhages. The *liver* weighed 110 Gm. and was dark brown in color. Beneath the capsule and on the cut surfaces there were irregular orange yellow areas which gave the parenchyma a geographic mottled appearance. These areas were demarcated from the surrounding brown parenchyma by narrow red zones. The consistency was somewhat variable, being softer in the orange-yellow areas. The *spleen* weighed 26 Gm. and was firm in consistency, chocolate-brown in color. On the cut surfaces, the follicles were somewhat obscured. The *blood vessels* were thoroughly explored, and no thrombi or other abnormalities were found. The *kidneys* appeared normal. The *brain* weighed 290 Gm., and there was a moderate excess of cerebrospinal fluid. On section, all the basal ganglia were dark yellow.

Microscopic description. The corpuscles of the *thymus* were numerous, but the staining of many was somewhat more pale than usual. They were infiltrated in many cases with polymorphonuclear leukocytes. Many of the reticulum cells showed nuclear pyknosis and karyorrhexis. Many of the alveoli of the *lungs* contained extravasated blood. There were also a few squames scattered throughout. The capsule of the *liver* was delicate. The architecture was grossly distorted by large areas of necrosis (Fig. 1) which corresponded to the orange-yellow areas seen in the gross specimen. In these areas the liver cells were, for the most part, completely destroyed. Hemopoietic foci within the necrotic areas could be distinguished, but the cells here also stained poorly. The necrotic areas appeared granular or fibrillar, and vascular channels, which could be identified, were empty. As one approached the periphery of a necrotic area, the liver cells showed varying degrees of necrobiosis. In some cells the cytoplasm was granular and appeared swollen, in others vacuolated, while the nuclei were pyknotic or showed karyorrhexis. In some places there were extravasations of blood. Also seen was a delicate reticulum of spindle-shaped fibroblasts and newly formed capillary buds which seemed to extend into the necrotic mass. In the relatively normal portions of the liver the usual architecture was fairly well maintained.

The liver cells contained brown pigment granules, and the Kupffer cells were prominent and also contained brown pigment granules. Scattered throughout were intrasinusoidal aggregates of deeply staining hematopoietic cells. The portal areas and vascular channels presented nothing of note. The malpighian follicles of the *spleen* were numerous, the sinusoids were engorged with blood and foci of hematopoiesis were scattered throughout. The *bone marrow* showed erythroid and myeloid hyperplasia. The cells lining the proximal convoluted tubules of the *kidney* appeared slightly swollen, and their cytoplasm was granular. Golden brown pigment granules were seen within many of the lining cells.

Central nervous system. Dr. I. M. Tarlov: "The most striking changes were seen in the nerve cells of the nuclear masses. The cell bodies were oval and the processes indistinct. The nuclei were shifted to a peripheral position and stained poorly, and many nuclei could not be differentiated clearly from the surrounding granular, poorly defined cytoplasm. Nucleoli were infrequently seen. Nissl granules stained intensely in isolated groups of neurons, but were absent in others. This degenerative picture was patchy in distribution, for in some places the nuclei were clearly defined and appeared fairly normal. The blood vessels were distended but were otherwise not remarkable."

The anatomic diagnoses were: massive necrosis in liver, kernicterus, hepatosplenomegaly, focal hemorrhages in lungs and thymus gland, necrosis of Hassall's corpuscles, cholemic nephrosis and hematopoiesis in liver, spleen and adrenal glands.

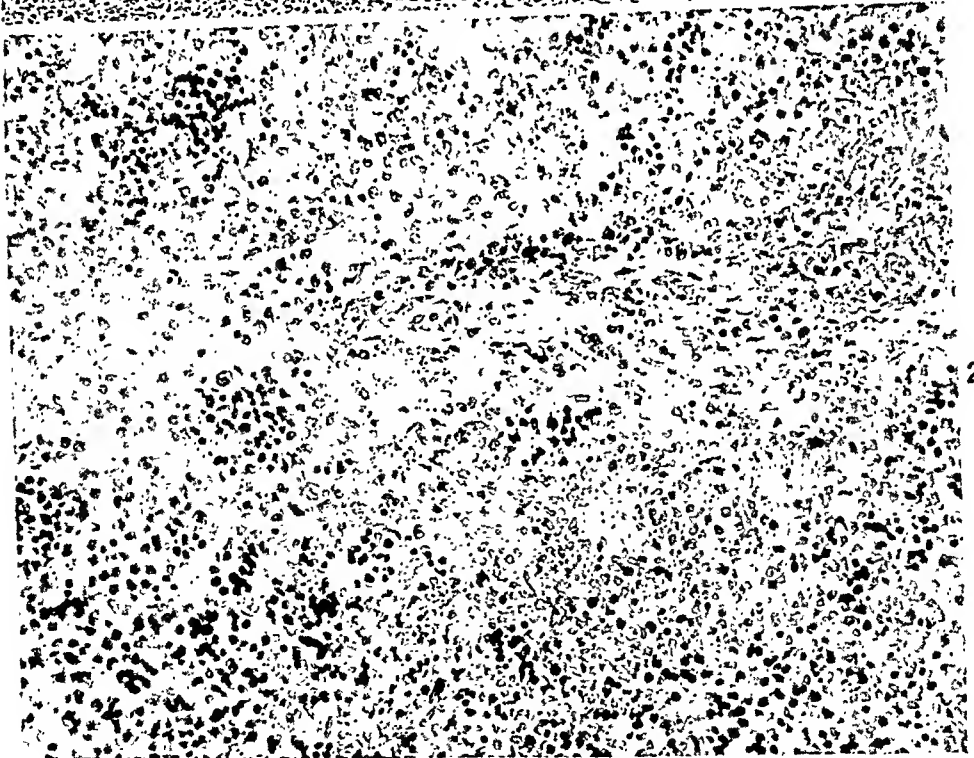
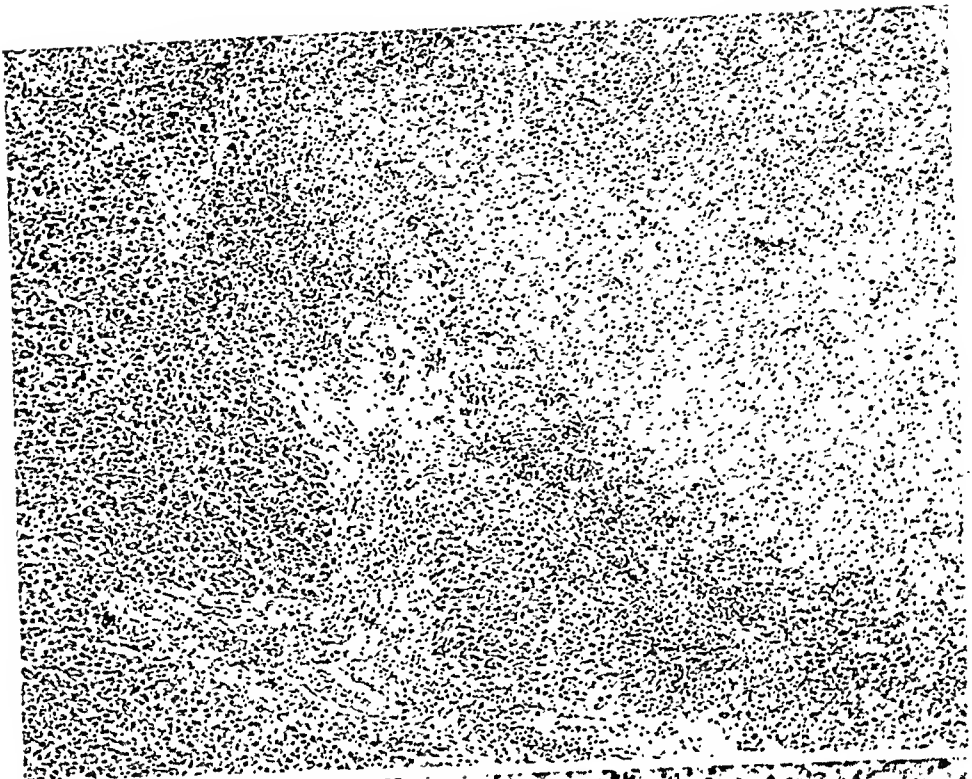
CASE 2

Clinical Data

J., a white female infant, was delivered by cesarean section on May 2, 1947. At birth her weight was 5 pounds 8 ounces (2.5 Kg.), and she appeared jaundiced and pale. The icterus index was 46, the hemoglobin 60 per cent and there were 102 nucleated red blood cells per 100 white blood cells. This baby was the issue of the mother's fourth pregnancy. The first pregnancy terminated in 1940 with the birth of a normal full term male infant that was neither jaundiced nor anemic. In 1942 she gave birth to a full-term female infant that developed jaundice. The infant was given one transfusion but died in thirty-six hours. A stillborn female was delivered two weeks before term in 1944. Blood grouping and Rh studies were performed on the family in January 1946, with the following results:

	Group	M-N Type	Rh-Irr Type
Father	O	M	Rh ₁ Rh ₂
Mother	A	M	rh
Son	A	M	Rh ₂

Studies of the mother's serum at this time showed the following: Agglutination test, negative; blocking test, 2 units; plasma conglutination test, 30 units. In October 1946, the mother, who had again become pregnant (last menstrual period September 5, 1946) was restudied. At this time the antibody titer was found to have fallen to 12 units by the conglutination method. The mother was treated with typhoid and pertussis vaccines, and periodic antibody titrations were done. On April 30, 1947, the titer, as determined by the plasma conglutination method, was 22 units. Because of this finding and because hydramnios had become manifest, the mother was subjected to cesarean section. The condition of the infant at birth was as noted above. The baby was Rh-positive (Rh₁rh) and belonged to group A. The cells clumped spontaneously when suspended in plasma, and free univalent antibodies were also present in the serum. An immediate exchange transfusion was done, using fresh bank blood from a group A, Rh-negative donor from which one-half the plasma had been removed and replaced with saline solution; 450 cc. of blood was administered while 380 cc. was drained. A total of 0.6 cc. of heparin was also given during the procedure; the amount of calcium gluconate given during the procedure was not stated. Following the transfusion, the icterus index was 30, the hemoglobin 63 per cent and the red blood count



(The sections in Figs. 1-8 were stained with hematoxylin and eosin.)

FIG. 1. Case 1. Liver showing massive necrosis. Note sharp demarcation from normal structure. $\times 80$.

FIG. 2. Case 2. Liver showing hemorrhagic necrosis. Note deeply staining erythroblasts in necrotic areas. $\times 350$.

3,900,000 per cu. mm. There were 164 nucleated red blood cells per 100 white blood cells. The liver edge was at the level of the umbilicus, and the spleen was palpable three finger-breadths below the left costal border. Respiratory efforts were weak and irregular. Two days after birth the infant was cyanotic, intensely jaundiced and was bleeding from the mucous membranes of the mouth. There were now 200 nucleated red blood cells per 100 white blood cells. The infant expired on the third day of life.

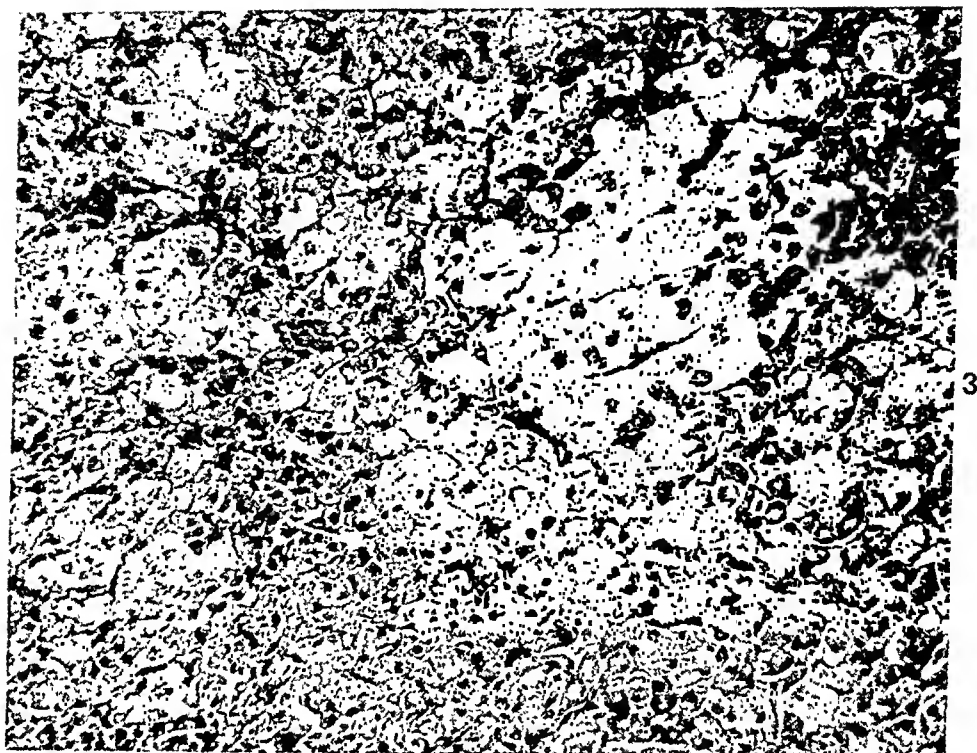
Necropsy Findings

Gross description. The body was that of a well developed, well nourished, white female infant, measuring 43 cm. in length and weighing 1760 Gm. The sclerae, *skin and mucous membranes* were icteric. There was no excess fluid in any of the serous cavities. The external and cut surfaces of the *lungs* showed scattered red areas of hemorrhage. The *liver* weighed 186 Gm. and was pale reddish brown with scattered yellow-gray mottling. The mottled areas were somewhat softer than the surrounding parenchyma. The *spleen* weighed 34 Gm. and was firm in consistency. On the cut surfaces the follicles were distinct. The *blood vessels* of the body were thoroughly explored, and no thrombi or other abnormalities were found. The adrenal glands and *kidneys* appeared normal. The *brain* weighed 454 Gm. The blood vessels were dilated. On section, the white matter appeared slightly yellow, the basal ganglia and dentate nucleus, bright yellow. No areas of hemorrhage were noted.

Microscopic description. In many places the alveoli of the *lungs* were completely filled with blood, and the bronchioles contained blood. Generally, the blood vessels were dilated and contained many nucleated erythrocytes. The capsule of the *liver* was delicate. There were small focal and large confluent areas of necrosis consisting of pink staining granular debris. Toward the periphery of the lobules, there were poorly defined liver cells with swollen granular pink staining cytoplasm which was often vacuolated. Nuclei also appeared swollen in some cells, pyknotic in others. Foci of hematopoiesis were scattered throughout. In the necrotic areas they showed less necrosis than the liver tissue (Fig. 2). There was no evidence of inflammatory reaction in any of the sections examined. Where the liver tissue was better preserved, the cells appeared finely granular and contained golden brown pigment granules within the cytoplasm. Pigment was also seen within prominent intrasinusoidal Kupffer cells and lying free in the stroma. Free pigment was especially prominent in the necrotic areas. Scattered throughout the *spleen* were foci of hematopoiesis. The sinusoids were congested. The follicles did not appear unusual. There was moderate hemosiderosis and erythrophagocytosis. The *bone marrow* showed moderate hyperplasia of the erythroid and myeloid elements.

The zona glomerulosa of the *adrenals* was intact. Within the deeper portions of the zona fasciculata and in the zona reticularis were small circumscribed foci in which the cells showed marked variation in size and shape. Many cells appeared swollen, and the cytoplasm was finely granular or reticulated in appearance (Fig. 3). Small vacuoles were also seen. Nuclei in many cells were very large, bizarre in shape, and chromatin material was eccentrically distributed. In some foci the cells were completely destroyed and appeared as granular pink and blue staining masses of detritus. An occasional hematopoietic aggregate of cells was also seen. There was mild cloudy swelling of the proximal convoluted tubules of the *kidneys* and brown pigment granules within the cells. Pink staining granular debris was seen within many of the lumens. Sections from various portions of the *brain* revealed varying degrees of degeneration of the nerve cells. The changes were similar to those seen in Case 1.

The anatomic diagnoses were: massive necrosis in liver, focal necrosis in adrenal glands, kernicterus, hepatosplenomegaly, hemorrhages in lungs, cholemic nephrosis and hematopoiesis in liver, spleen and adrenal glands.



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FIG. 3. Case 2. Adrenal gland showing focal areas of necrosis and cellular disorganization within the cortex. $\times 260$.

FIG. 4. Case 3. Liver showing massive pale necrotic areas. $\times 63$.

CASE 3

Clinical Data

Baby girl "L" was delivered by classical cesarean section on November 25, 1947, after thirty-six weeks of gestation. This baby was a sibling of "J. L." (Case 1). The mother became pregnant shortly after discharge from the hospital in 1946. The titer of her antibodies during her pregnancy remained at a level of from 10 to 15 units until the thirty-sixth week of pregnancy, when it suddenly rose to 50 units. Because of this, a cesarean section was promptly done. At operation the uterus was herniated, and there was retroplacental hemorrhage. At birth the baby was pale, its hemoglobin was less than 30 per cent and the red blood cell count about 1,000,000 cells per cu. mm. Fifty normoblasts per 100 leukocytes were seen in the smear. The baby was immediately given 120 cc. of group O, Rh-negative blood, and then an exchange transfusion was performed. In this procedure, 900 cc. of blood was given while 850 cc. of blood was removed. The baby had repeated bouts of cyanosis which were relieved by administration of oxygen during the transfusion. Calcium gluconate was given at frequent intervals during the operation (5 cc. of a 10 per cent solution for each 250 cc. of blood). At the termination of the exchange transfusion the hemoglobin was 88 per cent, the red blood cell count 5,900,000 per cu. mm., the leukocyte count 14,500 per cu. mm., and there were 124 nucleated red blood cells per 100 white blood cells in the blood smear.

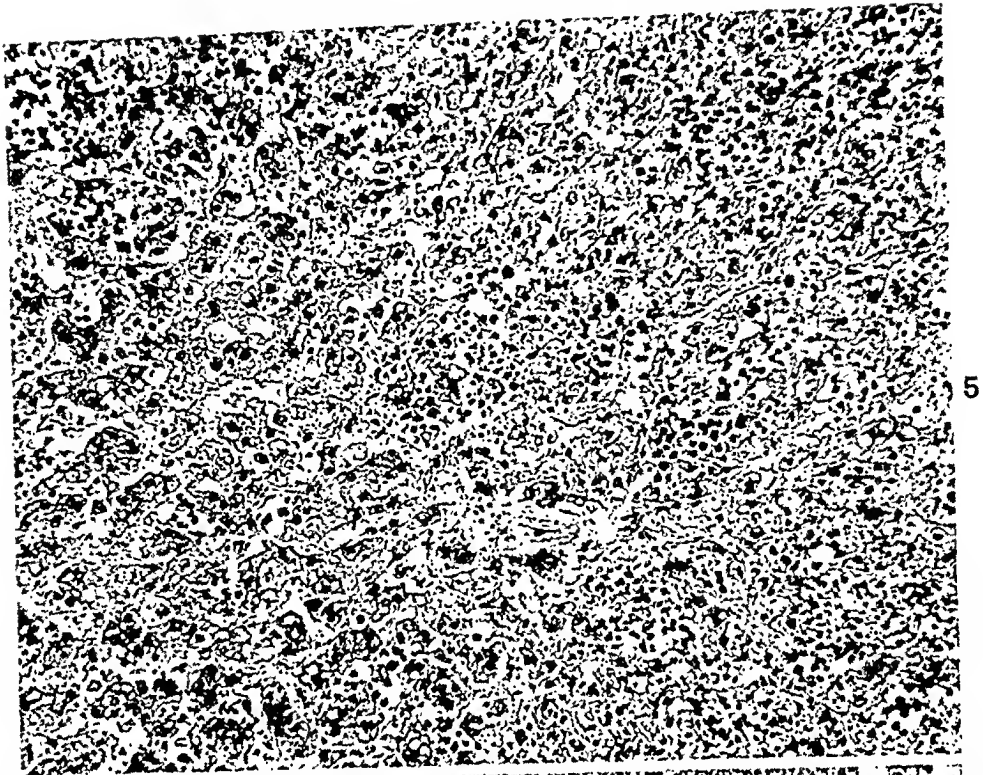
On examination the infant weighed 5 pounds 2 ounces (2.3 Kg.). Jaundice was absent, but the cord was stained yellow and the hands and feet were edematous. The liver was palpable 3 fingerbreadths below the right costal border, the spleen 1 fingerbreadth below the left costal border. The next day the baby breathed poorly, appeared lethargic and jaundice was evident. Reflexes were hyperactive and Chvostek's sign was present. The baby expired approximately twenty-five hours after birth. Blood taken immediately before death showed a calcium level of 7.2 mg. per 100 ml. The van den Bergh test showed the following: total bilirubin, 9.1 mg., and direct 0.8 mg. bilirubin per 100 ml.

Necropsy Findings

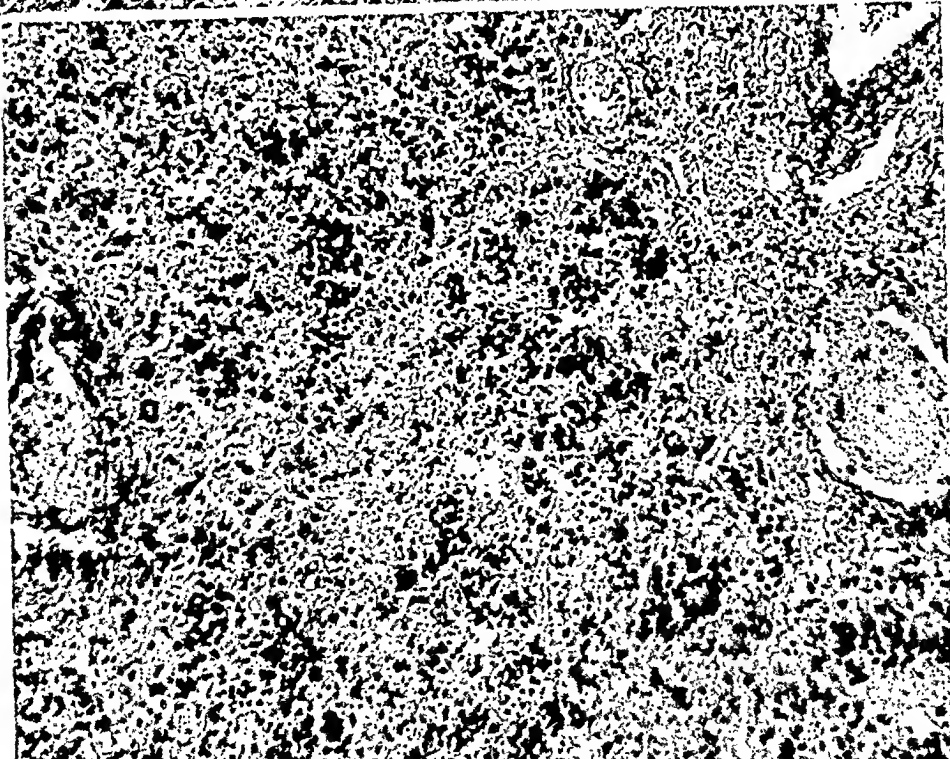
Gross description. The body was that of a well developed, well nourished, white female child measuring 46.5 cm. in length and weighing 2146 Gm. The sclerae, skin and mucous membranes were icteric. The serous cavities contained no excess of fluid. The *lungs* showed patchy areas of hemorrhage, especially at the bases. The *liver* weighed 171 Gm. The external surface was dark red-brown mottled with numerous geographic areas of orange-yellow interspersed with dark and bright red. The cut sections revealed orange-yellow areas with some hemorrhage. The *spleen* weighed 27.5 Gm., was firm in consistency, and on cut section the markings were indistinct. All blood vessels of the body were carefully explored, and no thrombi were found. The *brain* weighed 250 grams, its vessels were engorged and there was no evidence of kernicterus.

Microscopic description. Many of the alveoli of the *lungs* contained extravasated blood. The *liver* contained large areas of necrosis (Fig. 4). The liver cells stained poorly, and the cell cords were disorganized (Fig. 5). Many cells revealed no nuclei, and in other cells the nuclei were either shrunken and pyknotic or swollen. Scattered throughout were foci of blood and centers of hematopoiesis which stained fairly well (Fig. 5). The necrotic areas appeared similar to those described in the two previous cases, but the changes were less advanced. In addition, in one place, small venules were seen which were filled with fresh platelet thrombi (Fig. 6). The *spleen* contained numerous immature hemopoietic cells. In the zona fasciculata of the *adrenals* there were small irregularly rounded areas where the cells appeared swollen. The cytoplasm was granular or fragmented, and in some places the cells appeared to have dropped out of the supporting stroma (Fig. 7). No cellular reaction was evident. The *bone marrow* was hyperplastic.

The anatomic diagnoses were: massive necrosis of liver, focal necrosis of adrenal glands, hepatosplenomegaly, hemorrhages in lungs, and hematopoiesis in liver and spleen.



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FIG. 5. Case 3. Liver showing transition between relatively normal and necrotic areas. Note absence of inflammation and good preservation of hemopoietic cells. $\times 260$.

FIG. 6. Case 3. Area of necrosis in liver showing fresh platelet thrombi within blood vessels. $\times 260$.

DISCUSSION

It is well known that the liver in erythroblastosis fetalis is more profoundly affected than any other organ in the body, with the possible exception of the spleen¹⁸ and the basal ganglia. The changes, apart from the gross enlargement, are caused by the inability of the liver to excrete all of the bilirubin presented to it for elimination. Relatively minor histologic changes are the rule in livers of erythroblastotic infants, regardless of the severity or tenure of the disease. In some cases, when hyperbilirubinemia overtaxes the capacity of the liver, the bile capillaries appear loaded with pigment and excessive amounts are also seen within the liver cells and Kupffer cells. Rarely, a mild to moderate degree of fatty change is seen. In addition, the hepatic cells may show cytoplasmic granularity or some degree of swelling. These changes almost never become extreme except where postmortem autolysis may have played a role. In addition, there are, of course, the usual disseminated foci of hematopoiesis which are variable in number and extent. It must be noted, however, that the anatomic findings may not accurately indicate the extent of impairment of physiologic functions.

In the three cases described above, the usual findings of erythroblastosis fetalis were seen in the relatively "normal" portions of the liver parenchyma. Superimposed upon this was a most dramatic and explosive episode which resulted in the simultaneous death of large portions of the hepatic parenchyma. Polayes¹⁵ in 1942, mentioned extensive destruction of the liver architecture in infants with icterus gravis who lived a week or more. It is highly doubtful, however, that this was similar to the areas of massive necrosis described above.

At this point it is pertinent to speculate as to the actual cause or causes of the lesions found. For purposes of clarity, the substitution transfusion will be discussed under the following headings:

A. The erythroblastotic infant

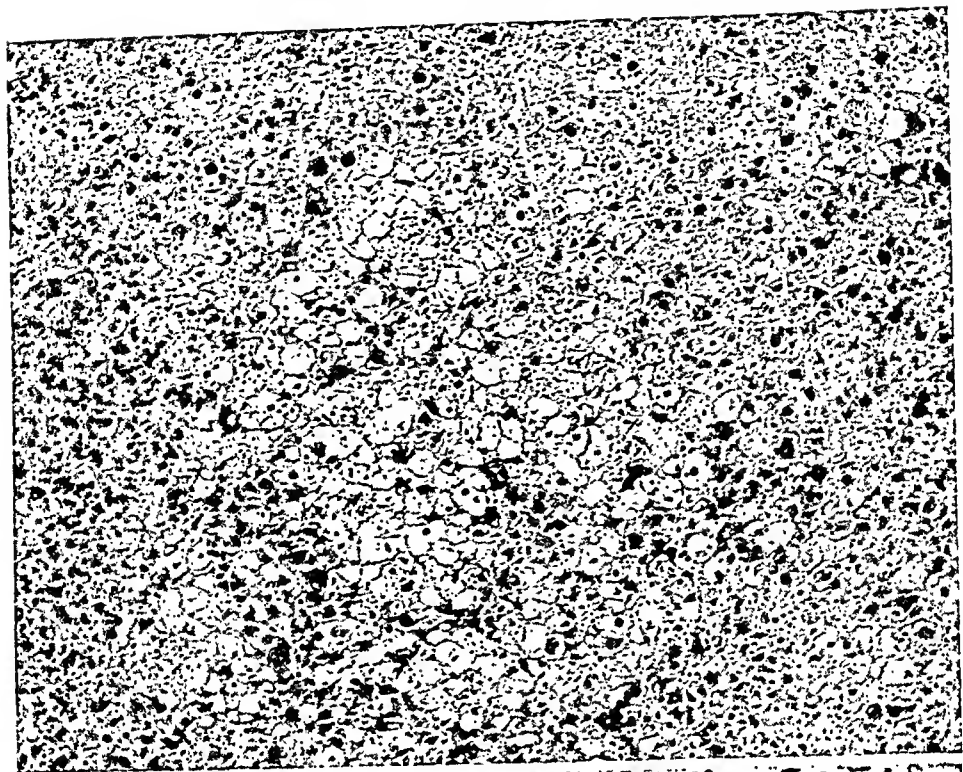
B. Factors concerned with the transfusion:

1. Blood
2. Sodium citrate
3. Calcium gluconate
4. Rate of administration
5. Heparin

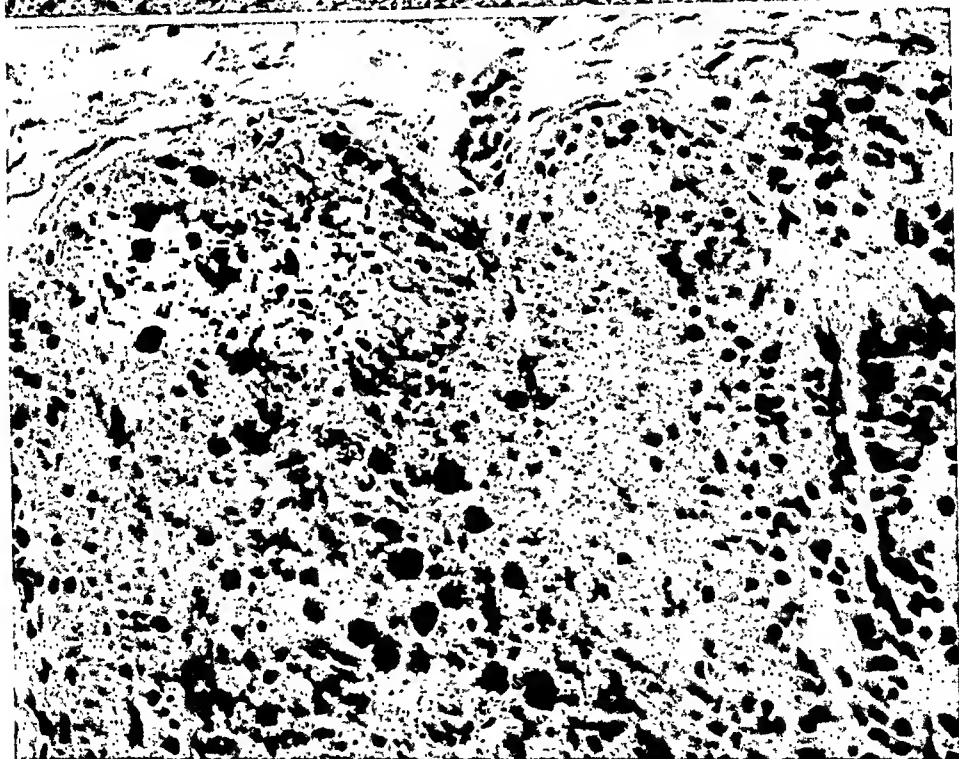
A. The Erythroblastotic Infant

The infant born with erythroblastosis fetalis suffers from an illness which is usually the result of an Rh incompatibility with the mother. Ordinarily, the infant is afebrile, and if it does not have symptoms at the time of birth, it rapidly develops some or all of the following symptoms or findings: jaundice, anemia, weakness, edema, a hemorrhagic tendency, hepatosplenomegaly and erythroblastemia.

Damage to the erythroblastotic infant's liver may very well result from products of blood hemolysis, such as hematin and bilirubin. Thus, accord-



7



8

FIG. 7. Case 3. Adrenal gland showing focus of cellular swelling and necrosis. $\times 80$.

FIG. 8. Case 4. Adrenal gland showing coagulation necrosis in cortex. $\times 520$.

ing to Davidsohn,⁵ hemolytic anemia due to the action of Rh antibodies is not only the initial cause of erythroblastosis, but it explains the complex manifestations of the disease as well.

Dameshek, Greenwald, and Tat⁴ have demonstrated an increased excretion of breakdown products of hemoglobin in cases of erythroblastosis fetalis, thus providing direct evidence in favor of the hemolytic nature of the disease. Hill and Haberman⁹ recently described actual hemolysis of Rh-positive cells *in vitro* by the Rh antibody, as did Diamond and Abelson⁶ in their slide test in which a heavy suspension of erythrocytes is used. Indirect evidence is afforded by many of the clinical and anatomic findings such as the anemia, erythroblastotic hyperplasia of the bone marrow and extra-medullary hematopoiesis.

There are those who believe that degradation protein products of hemolysis, apart from the effects of hyperbilirubinemia and excessive pigmentary deposition, may cause further specific cellular damage. For example, Wallerstein²¹ has suggested that a toxin, possibly arising from the stroma of lysed cells, contributes to the fatal outcome in many cases. It is also believed by some that the liver of the newborn infant is functionally immature and, consequently, is unable adequately to detoxify, or otherwise take care of, all the hemolytic fractions produced in erythroblastosis fetalis.²²

Recently, Wiener and Brody²³ described agglutinative thrombi in the brain of an erythroblastotic infant who showed kernicterus. It is their belief that many of the clinical features of the disease are due to such intravascular clotting. However, there is little to favor this concept, enticing though it may be from a theoretical point of view. The anatomic studies of Javert,¹¹ Ferguson,⁷ Potter,¹⁷ and Follis, Jackson and Carnes⁸ also fail to give support to this theory. Personal consultation with other pathologists, such as Polayes¹⁶ and Lederer,¹² are also non-contributory. The author reviewed all of the cases of erythroblastosis fetalis in which the infant came to autopsy at the Jewish Hospital since 1936 and failed to find agglutinative red blood cell thrombi. The thrombi seen in Case 2 (Fig. 3) were composed of platelets and appeared to be related to the necrotic process in the liver, rather than to the hemolytic disease. It is, therefore, believed that thrombosis may be safely dismissed as a factor in the pathogenesis of erythroblastosis fetalis.

In summary, the erythroblastotic infant is a sick infant in whom active hemolysis plays a major role in pathogenesis. Its liver is also functionally immature and has difficulty in coping with the degradation products of the hemolytic process. In some instances, prematurity may serve as a further handicap in treatment.

B. Factors Concerned with the Transfusion

1. *Blood.* The blood used in the transfusions was Rh-negative, type specific and compatible. The amount used varied from 500 to 1000 cc. (one to two units), and approximately a 90 per cent exchange of the infant's blood was effected, as shown by differential agglutination tests. Why this amount has been so universally adopted as optimum is not clear to the author. For the

present, however, it is probably safe to say that the transfused blood *per se* caused no damage. It is, however, possible that the total amounts used may be excessive.

2. *Sodium citrate.* The literature contains many contradictory reports concerning the toxicity of large amounts of citrated whole blood. Wiener²⁵ *et al.* report the administration of 5500 cc. and 7000 cc. of citrated blood to two adult patients with recovery. On the other hand, Ivy and his associates,¹⁰ on the basis of experimental work, concluded that 1500 to 2500 cc. of citrated whole blood given rapidly to a man weighing 70 Kg. might prove fatal.

Bruneau and Graham³ reported experiments in which dogs were bled 10 per cent of their estimated blood volume and the blood then re-injected during a one-half hour period. The re-injected blood contained either 0.6 per cent sodium citrate or heparin; bleedings and replacements were repeated until the animals died. The dogs receiving the citrated blood expired after an average of 9.25 bleedings, or after a total of approximately 92.5 per cent of the estimated blood volume had been withdrawn and re-injected, while the dogs receiving heparinized blood died after an average of 25.2 bleedings, or after a total of approximately 252 per cent of the estimated blood volume had been withdrawn and re-injected. Moreover, the dogs given citrated blood showed before death definite toxic symptoms not seen in the other animals.

Adams and his co-workers¹ repeated Ivy's experiments. They agreed that citrated blood given rapidly in the treatment of shock produced by hemorrhage resulted in the death of the animal. When bleeding and transfusions were conducted simultaneously, an average of 122 per cent of the estimated blood volume was exchanged during a period of seventy minutes before the animal expired. When the rates of bleeding and transfusion were less rapid, a larger volume was exchanged without reaction.

Thornton *et al.*²⁰ performed exchange or substitution transfusions on dogs, using heparin or citrate as anticoagulants. In their experiments with citrate, calcium gluconate was used to restore calcium ions. These experiments were very similar to the exchange transfusions given for erythroblastosis. It was found that the citrated animals invariably died much sooner than those given heparinized blood. Thornton and his associates attributed death in these animals to the excess of fluid added to the circulating blood. They did not describe any changes in the liver.

If one returns to the statement of Ivy that 1500 to 2500 cc. of citrated whole blood given rather rapidly to a 70 Kg. man may prove fatal, it might be well to calculate just how much sodium citrate is actually given under such circumstances. Since it is customary to use 50 cc. of a 3.8 per cent solution of sodium citrate for each 500 cc. of blood, the dosage in Ivy's hypothetical case would be of the order of 0.08 to 0.13 Gm. sodium citrate per Kg. of body weight. Assuming that the infants have an average weight of 2.7 Kg., the dosage of sodium citrate given during the exchange transfusion is of the order of 0.7 to 1.4 Gm. sodium citrate per Kg. of body weight, or approximately ten times per Kg. of body weight as in an adult receiving from 1500 cc. to 2500 cc. of citrated blood.

It would seem that this amount of sodium citrate represents a tremendous dose which a liver already damaged by the erythroblastotic process may be required to detoxify or otherwise metabolize. If this is true, it may be necessary to limit the amount of sodium citrate used in substitution transfusions.

In experiments¹⁹ involving perfusion of the intact liver of healthy animals with citrate, very little citrate ion remains in the perfused fluid after its passage through the liver, indicating that the citrate is very quickly metabolized by the normal liver. The lack of liver findings in experimental animals mentioned above does not necessarily invalidate this assumption since the animals were healthy and, furthermore, since they died within a few hours of the termination of the operation, there may not have been sufficient time for the evolution of the lesions in the liver.

3. *Calcium gluconate*. During the exchange transfusion, 5 to 10 cc. of 10 per cent calcium gluconate is injected intravenously rather rapidly in fractional doses to replace calcium ions bound by the citrate solution. Ordinarily, calcium gluconate is considered an innocuous drug, and its value in tetany, except for a transient effect, is doubtful.² Aside from its inhibitory effect on the cardiac musculature, little is known concerning its toxic potentialities. For an adult weighing 70 Kg., the injection of 5 to 10 cc. of the 10 per cent solution represents a dose of 7 to 14 mg. calcium gluconate per Kg. of body weight. On the other hand, the average infant weighing 2.7 Kg. receives 180 to 370 mg. calcium gluconate per Kg. of body weight, which represents a dosage 25 times that commonly used in adults. The toxicity of such a relatively large dose should be studied.

Additional evidence of the toxic potentiality of calcium gluconate is afforded by an unique case which is presented by permission of Dr. Nathan Mitchell, Pathologist to the Beth-El Hospital, Brooklyn, New York.

CASE 4

Clinical Data

After an uneventful pregnancy, a 30 year old mother, gravida II, para I, was delivered of a term infant by low forceps. Her serology was negative, and her blood cells belonged to group O and were Rh-positive. At the time of birth, the infant was in fair condition, but artificial respiration was required and two ampules of eoramine were administered. No untoward symptoms were noted until the third day when a temperature of 101 F. was observed. At this time 10,000 units of penicillin were injected, and the dose was repeated every three hours; but, the temperature rose to 104 F. On the fourth day of life there was a suggestion of carpopedal spasm, and the infant was, therefore, given calcium gluconate. The baby died on the eighth day despite continuous intravenous fluids and other supportive measures. The calcium gluconate did not materially change the spastic phenomena, and toward the end there was respiratory difficulty. A total of a little over eleven ampules (11.3 Gm.) of the 10 per cent solution of calcium gluconate was given in all. Jaundice was noted on the last two days of hospitalization.

Necropsy Findings

At autopsy, no evidence of infection was found in any of the viscera. There were no stigmata of erythroblastosis. The liver was studded with innumerable tiny focal areas

of coagulation necrosis which were more frequently seen in the mid-zonal portions of the liver lobule, although other areas were not spared. Focal areas of necrosis were also seen in the cortex of adrenal glands (Fig. 8).

Comment

In this case, the cause of the baby's fever initially was obscure. Experience with penicillin to date has been extensive enough to preclude effectively this antibiotic as a cause of necrotic lesions. Infectious hepatitis was also eliminated, since neither mother nor child received blood or plasma, and the mother had no illness during the course of her pregnancy. The only apparent noxious agent was the calcium gluconate which, obviously, had been given in heroic and excessive dosage.

The similarity in the lesions of this infant and of the three infants with erythroblastosis previously described is striking. All showed coagulation necrosis of the liver and adrenal glands as predominant findings. However, the erythroblastotic infants exhibited massive areas of hepatic necrosis, whereas in Dr. Mitchell's case, the lesions were discrete and the foci were minute.

4. *Rate of administration.* It has been noted above that giving of an exchange transfusion consumes approximately ninety minutes. Without discussing the question of speed shock, it may be mentioned that in the experimental work on dogs quoted previously, all the investigators stated that the amount of time consumed administering the transfusion is very important. Specifically, the number of bleedings and replacements could be materially increased in the experiments with either citrate or heparin if the procedure were slowed.

In the damaged liver of the erythroblastotic infant, it is probable that the citrate is not handled as efficiently as under normal circumstances and that a rapid transfusion may very well overtax its limited capacity with catastrophic results.

What has been said for sodium citrate may also apply to calcium gluconate. The effect of rapid administration may be damaging, and it, therefore, seems at least for the present, that substitution transfusion should be conducted at a slower rate.

5. *Heparin.* Heparin is the natural anticoagulant of the body. When it is given intravenously, there is a rapid prolongation of the coagulation time followed by a precipitous drop to or below the normal level. When given intravenously in a single dose or in fractional doses, the injection of 5 cc. or 50 units is not considered excessive for an average adult. For a person weighing 70 Kg., this represents 0.7 units of heparin per Kg. of body weight. The dose given the infant was 0.2 cc. or 2 units, which also represents approximately 0.7 units per Kg. of body weight. In other words, the dosage of heparin given the infants is proportional to that given to adults.

The author¹³ has given much larger doses of heparin to patients suffering from subacute bacterial endocarditis or thrombophlebitis without deleterious results. Heparin has proved to be quite safe if proper precautions in its use are taken. For these reasons, it is believed that heparin probably played no significant, if any, role in the production of the necrotic lesions in the liver and adrenal glands. Hemorrhage, not necrosis, is characteristic of heparin overdosage.

SUMMARY AND CONCLUSIONS

This report concerns the finding of liver necrosis in three infants with erythroblastosis fetalis and in one infant with calcium gluconate intoxication. In the cases of erythroblastosis, the diagnosis was made antenatally by means of serologic tests, the pregnancies were terminated by cesarean section before term, and the babies were immediately transfused by the exchange or substitution technic. The infants lived from one to five days; at necropsy, all showed massive liver necrosis, and two of the infants also showed focal necrotic lesions in the adrenal glands. These changes were attributed to blood transfusions. It is considered that a combination of the following factors may have been responsible for these untoward findings: (1) the presence of erythroblastosis; (2) the administration of excessive amounts of sodium citrate and/or calcium gluconate in the transfused blood; and (3) excessive speed of administration of the transfused blood.

While the exchange transfusion may be a valuable adjunct in the treatment of erythroblastosis fetalis, in view of the lesions described above, it must be considered an unsafe procedure as given at the present time. Consequently, one should carefully consider all the indications for the procedure and use it with caution. The use of heparinized blood, rather than citrated blood, is suggested, since this would make unnecessary the use of either sodium citrate or of calcium gluconate. It is also suggested that the rate of effecting the exchange transfusion be slowed so that the infant may be enabled to adjust itself to the procedure.

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ON THE SIGNIFICANCE OF Hr SENSITIZATION IN Rh ANTIBODY DETERMINATIONS*

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The Hr blood factor was encountered by Levine and co-workers^{3, 4} early in their studies on the pathogenesis of erythroblastosis fetalis. This blood agglutinin was designated as Hr because of its reciprocal relationship to the Rh factor. Isoimmunization to Hr was shown to account for certain cases of erythroblastosis where the mother was Rh-positive and developed antibodies which agglutinated Rh-negative erythrocytes as well as many Rh-positive blood specimens. The Hr factor is generally considered to be a poorer antigen than Rh since instances of Hr sensitization are quite rare even though the incidence of Hr-negative persons is comparable to that of Rh. Experience in this laboratory justifies the common belief that tests for Hr sensitization are of little clinical value in routine Rh examinations. Only one anti-Hr serum has been encountered in more than five years of testing. This was demonstrated with a postpartum blood specimen obtained from the mother of erythroblastotic twins. Since the Hr factor is thought to be of feeble antigenicity, the sensitization of this woman is noteworthy for it occurred in her first pregnancy and there was no evidence that transfusions or intramuscular injections of blood were predisposing factors.

A white woman, aged 22, delivered twin boys normally, in spontaneous labor, two months prematurely at the Physicians and Surgeons Hospital, Glendale. This had been her first pregnancy, and she had had no abnormal signs or symptoms during gestation. Her past medical history contained nothing apparently relevant. She had been hospitalized two days as a child because of swallowing a pin, but on repeated questioning there could be obtained no history of any kind of blood injection at that time. This information was corroborated by her mother. Physical examination showed no significant abnormalities. The blood Wassermann test was negative.

The puerperium was normal. (Three months later the patient was again pregnant.)

Twin No. 1 weighed 4 lb. 3 oz. (1930 Gm.), and showed marked edema of extremities and scrotum. He required resuscitative measures, remained in poor condition and died after eight hours.

Twin No. 2 was born after an interval of ten minutes and appeared in somewhat better condition. He weighed only 2 lb. 4 oz. (1060 Gm.) but cried and breathed spontaneously. He showed no abnormalities other than those of prematurity. There was no edema, and no recognized icterus during life. His blood contained 1.4 million erythrocytes and 14,000 leukocytes per cu. mm. Hemoglobin was 7.8 Gm. per 100 ml. There were 563 nucleated red cells for each 100 leukocytes.

* Received for publication, June 17, 1948.

His condition rapidly deteriorated, with cyanosis and difficult breathing, and he died twenty hours after birth.

POSTMORTEM FINDINGS

(Autopsies performed by Dr. Albert W. Brown)

Twin No. 1 was 41 cm. long, and showed pallor, abdominal distention, scrotal edema and areas of confluent petechial hemorrhages. There was no icterus.

Internal examination revealed small quantities of transudate in the pleural and peritoneal spaces. The liver and spleen were enlarged to relatively enormous dimensions, both extending nearly to the symphysis. The spleen was soft and easily ruptured, liberating semi-liquid slate-red pulp.

Microscopic examination of the organs showed a high proportion of nucleated cells in the intravascular blood. In the liver there was massive hematopoiesis accompanied by advanced degeneration of the hepatic cells. Moderate hemosiderosis but no bile retention was present. The spleen showed marked active hematopoiesis, and a peculiar ferruginization of the capsule and trabeculae.

Diagnosis: Erythroblastosis fetalis, hydrops type.

Twin No. 2 differed from the first twin in being only 35 cm. long, having no edema or petechial hemorrhages, and showing moderate but definite icterus. The jaundice was also noted grossly in the liver and in the vascular intima, but not in the brain. The liver and spleen were only slightly enlarged.

Microscopic examination showed marked active hematopoiesis in liver and spleen, and a high proportion of nucleated cells in the vessels of the lungs. The hepatic structures were fairly well preserved and showed much hemosiderosis, as well as a moderate amount of bile pigment.

Diagnosis: Erythroblastosis fetalis, icteric type.

The placenta was also examined. It was moderately pale and appeared to be of the single-ovum type, with two amniotic cavities. Microscopic examination of each side of the placenta showed large numbers of nucleated cells in the fetal blood.

Blood typing studies (by Roy T. Fisk, Ph.D.) gave the following information:

Mother, group B, Rh₁Hr'-negative

Husband, group AB, Rh-negative Hr'-positive

Infant 1, group A, Rh₁Hr'-positive

Infant 2, group A, Rh₁Hr'-positive

The mother's serum contained anti-Hr' agglutinins in a titer of 1:32 when tested by the saline agglutination method with group O, Rh₁Hr'-positive erythrocytes, and a titer of 1:64 resulted with the gelatin conglutination method of testing.² Group O, Rh₁Hr'-negative erythrocytes gave no reaction with the serum. A second serum specimen which was obtained four months later showed a greatly reduced agglutinin titer of 1:2, but the conglutination test remained positive in a serum dilution of 1:64. Anti-Hr' agglutinins were also demonstrated in a specimen of breast milk, but positive reactions could be obtained only with the undiluted milk.

Washed erythrocyte suspensions made from postmortem blood specimens of the twin infants were shown to be sensitized by the antihuman globulin test of Coombs *et al.*¹ and by the gelatin agglutination method. Antibodies of anti-Hr' specificity were eluted from the saline washed erythrocytes by heating the suspensions at 50 C. for 10 minutes and centrifuging while hot. Weak reactions were also obtained from the postmortem serum specimens of the infants. In both instances, the eluates or whole serums produced clumping in the presence of gelatin but not when saline alone was used. The reactions were specific for Hr'-positive cells.

Rh_o-positive women seldom develop atypical iso-antibodies as a result of pregnancy. A series of 1767 unselected serum specimens from such women was examined for antibodies which might react with Rh-negative bloods using the sensitive gelatin antibody exclusion test. Approximately 350 of these women would be expected to be Hr'-negative and an additional 50 might be Hr''-negative. By comparison 382 routine specimens from Rh_o-negative women were tested by the same method against Rh_o-positive bloods. None of the 1767 Rh_o-positive serums gave evidence of reacting with the Hr-containing test bloods while 22 or 5.7 per cent of the 382 Rh_o-negative women showed positive Rh antibody tests. The failure to detect Hr antibodies among the series of Rh_o-positive serums might be expected in view of the rarity of clinical erythroblastosis resulting from sensitization to this blood factor.

SUMMARY

Sensitization to the Hr factors is so uncommon compared to Rh that routine Hr typing of pregnant women is not necessary. However, evidence has been presented that the Hr' factor is not necessarily a poor antigen. This report is concerned with a mother who became sensitized during her first pregnancy without history of previous exposure to the antigen. In this case the Hr antibodies led to the severest types of erythroblastosis. It is of interest that the twin infants developed pathologically dissimilar forms of this condition, although presumably being subjected to the same maternal antibody.

Acknowledgments. Valuable technical assistance was provided by Catherine A. McGee, B.S. Specimens of blood from the mother of the erythroblastotic twins were obtained through the courtesy of A. W. Allum, M.D., and D. R. Richard, M.D.

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BOOK REVIEWS

Clinical Toxicology, Ed. 2. By CLINTON H. THIENES, M.D., Ph.D., Professor of Pharmacology and Head of the Department of Pharmacology and Toxicology, School of Medicine, University of Southern California, Los Angeles, and Attending Pathologist (Toxicology), Los Angeles County Hospital; and THOMAS J. HALEY, Ph.D., Fellow in the Department of Pharmacology and Toxicology, School of Medicine, University of Southern California, Los Angeles. 373 pp., 9 figs., 9 tables. \$4.75. Philadelphia: Lea and Febiger, 1948.

This book is "written primarily as a classroom text and as a guide for the general practitioner". Its subject matter falls into four general headings. The first concerns the toxicology of the various poisons and consists of six sections covering in order, the convulsants, the central nervous system depressants, the peripherally-acting nerve poisons, the muscle poisons, the general protoplasmic poisons and poisons which affect the hematopoietic system. Section 7 discusses the treatment of poisons, which includes measures to decrease absorption, the elimination of absorbed poison, physiologic antagonism of poisons and, finally, a page on the general care of victims of poisoning. The third portion of the book presents an outline of symptom diagnosis. Here are listed under the headings of various symptoms and signs encountered in the emergency room, the toxic agents capable of producing the symptom in question. While the general nature of poisons does not allow the section to be of outstanding value in arriving at the diagnosis in a given case, it does serve to recall to mind the less well-known members of a group of different poisons capable of producing the same symptoms. The final 105 pages of the book are devoted to chemical methods of detecting the various toxic agents. The tests, while not new or radically different from those in earlier texts, are comprehensive and the technics in this portion of the book are, for the most part, accompanied by references to the literature. Since, as is characteristic of toxicologic analysis, many of the technics are qualitative in nature and are based on color or biologic tests, the reader is warned that the identification of a poison "requires usually that it be obtained in a relative state of purity in order that it give its specific color, crystal or precipitation reactions with the test chemicals."

In general, the format is good. There are few typographic errors, and the subject matter is covered in a concise and sufficiently detailed nature to be of real value to student and general practitioner, as well as, on occasion, to the practicing toxicologist. This reviewer regards the book as one of the most pleasing manuals to appear in recent years and recommends it highly.

Boston

R. S. FISHER

Pathological Histology, Ed. 3. By ROBERTSON F. OGILVIE, M.D., F.R.C.P. (Edin.), F.R.S.E. Lecturer in Pathology and Assistant in Forensic Medicine, University of Edinburgh; Senior Pathologist, Royal Infirmary, Edinburgh; Pathologist to the Leith and Deaconess Hospitals, Edinburgh; Examiner in Pathology and Forensic Medicine for the Triple Qualification. 459 pp., 260 photomicrographs in color. \$10.00. Baltimore: The Williams & Wilkins Company, 1947.

Previous editions of this book appeared in 1940 and 1943. The preface states that it is intended to be a companion volume to the standard textbooks of pathology, to be used by medical students or by graduate students wishing more knowledge of histologic pathology. Like most textbooks of pathology, it is divided into two sections, the first taking up general pathologic processes, and the second describing the pathology of the various systems of the body. Under each heading there is a short introductory paragraph which is followed by gross and microscopic descriptions of the pathologic processes. The book is illustrated by 260 excellent photomicrographs in color which have been reproduced by the Finlay process. The latter have been chosen with care, and their reproduction is excellent. Beneath each photograph there is a short note describing the essential features illustrated. Common

staining reactions of various tissues are well described. The index appears to be adequate.

The author's style is clear and concise but, as may be expected, much of the terminology is somewhat different from that used in the United States. For instance, the author uses the term endothelioid, rather than epithelioid; syphilis, he states, is due to the *Spirochaeta pallida* rather than *Treponema pallidum*; tumors are divided into simple and malignant tumors rather than benign and malignant, and papilliferous cystadenomas of the ovaries are described as papilligerous. In many places there appears to be little logical arrangement of the subject matter and, naturally, in such a book there is little continuity between the various subjects described. It may seem strange to an American reader that malignant tumors are divided into three groups: sarcoma, carcinoma and endothelioma. The only example of the latter which is described is the synovioma which is usually considered to arise from mesothelium rather than from true endothelium. On Page 150, it is stated, "Microscopically, the synovioma is peculiar in that it is apparently made up of mixed sarcomatous and carcinomatous tissues." Mixed tumors are stated to arise from pleuri-potential cells and under this heading are included mixed tumors of the parotid. Again, on Page 234, it is stated that the absorption of the by-products of young pin worms may cause "characteristic helminthic toxæmia". A student might receive a wrong conception of the importance of some of the diseases described; for example, three pages are devoted to Reidel's struma of the thyroid, whereas only one page is devoted to the entire pathology of the pituitary gland, only acromegaly with eosinophilic adenoma being described.

It is doubtful whether many pathologists would recommend that their students purchase this book in addition to another required text in pathology, or that a pathologist would find much use for this book for his own needs, since it is not comprehensive and contains no bibliography. However, it should form a valuable addition to the libraries of those institutions in which postgraduate students have need of short, concise histologic descriptions of the more important pathologic processes.

Dearborn, Michigan

H. J. LINN

Clinical Diagnosis by Laboratory Methods, A Working Manual of Clinical Pathology, Ed. 11.

By JAMES CAMPBELL TODD, PH.B., M.D., Late Professor of Clinical Pathology, University of Colorado School of Medicine; and ARTHUR HAWLEY SANFORD, A.M., M.D., Professor of Clinical Pathology, Mayo Foundation, University of Minnesota and Senior Consultant, Division of Clinical Laboratories, the Mayo Clinic; with the collaboration of GEORGE GILES STILWELL, A.B., M.D., Division of Clinical Laboratories, the Mayo Clinic. 954 pp., 397 figs. \$7.50. Philadelphia and London: W. B. Saunders Company, 1948.

The eleventh edition of this popular text has been completely rewritten and re-organized. It includes many significant additions to clinical pathology which were made during the five years since the last edition. There are, however, several tests, particularly in the section on clinical chemistry, that have not been described, *e.g.*, the thymol turbidity and flocculation tests, the intravenous hippuric acid and the intravenous glucose tolerance tests. The exact technic for the prothrombin test with response to vitamin K is not given. The discussion of the principles of the tests, the sources of error and interpretations of results are, generally, far too brief. While the American literature on the Rh factor is adequately reviewed, no mention is made of the Fisher-Race theory and classification. These few omissions do not detract significantly from the exemplary general character of this textbook.

The black and white prints and the illustrations in color are excellent and unusually well chosen. Because of the concise and clear presentation of the many practical methods, this book will be valuable to those who make either occasional, frequent or constant reference to clinical pathological procedures. It will continue to serve as one of the leading and most useful texts on clinical pathology.

Manual for Laboratory Work in Mammalian Physiology. By FRED E. D'AMOUR and FRANK R. BLOOD. 84 pp., 50 experiments (looseleaf). \$2.75. Chicago: The University of Chicago Press, 1948.

Manual for Laboratory Work in Mammalian Physiology by D'Amour and Blood is unique in outlining a fairly complete set of physiologic experiments to be performed exclusively on rats. The demonstration of the suitability of the laboratory rat for most routine laboratory experiments should relieve many departments of physiology of the expense and trouble of maintaining larger and less easily propagated animals.

The manual contains many helpful suggestions on the care and maintenance of a rat colony and the few items of special equipment necessitated by the size of the rat are adequately described and in most instances illustrated by photographs. The techniques are relatively simple and for the most part could be executed with the equipment supplied by the average physiologic laboratory. It should be equally helpful to students and instructors.

Detroit

WILHELMINA F. DENNING

Hemorrhage. By GREGORY SHWARTZMAN, C. H. BEST, ROBERT CHAMBERS, CHARLES S. DAVIDSON, JOHN H. FERGUSON, ROBERT F. FURCHGOTT, I. E. GERBER, MAGNUS I. GREGERSEN, PAUL GYÖRGY, RUSSELL L. HADEN, C. HYMAN, L. B. JACQUES, PAUL KLEMPERER, R. E. LEE, C. N. H. LONG, DICKINSON W. RICHARDS, JR., R. H. SCHNEIDER, EPHRAIM SHORR, HENRY J. TAGNON, S. A. THAYER, LEE C. UNDERWOOD, DONALD D. VAN SLYKE, ALFRED E. WILHELM, and B. W. ZWEIFACH. 178 pp., 53 figs., 21 tables. \$3.00. New York: Annals of the New York Academy of Sciences, 1948.

The monograph integrates on a broad basis recent experimental viewpoints of many different investigators and thus serves as a valuable source of information and a foundation for further progress. It is stimulating and profitable reading from cover to cover. There are included discussions by competent workers which followed the systematic presentations by the essayists listed above. The results of generous support offered to investigators during World War II are in part reflected in the advances summarized in this monograph.

The scope of the book can, perhaps, be indicated best by citing the topics covered. In blood coagulation there is a review of basic facts. There are presentations on heparin in thrombosis, vitamin K and the clinical aspects of hypoprothrombinemia. The effects of hemorrhage on circulation, peripheral blood vessels and the kidney are considered. Other contributions include studies on experimental deficiencies of pantothenic acid, choline and cystine; traumatic and hemorrhagic shock; vasomotion in the hemodynamics of the blood capillary circulation; hepatorenal factors in circulatory homeostasis; metabolic changes associated with hemorrhage; hemorrhagic manifestations of bacterial and virus infections, and abnormal hemorrhage with normal platelet count and normal clotting.

Detroit

WALTER H. SEEGER

Coronary Heart Disease. By A. CARLTON ERNSTENE, M.D., Chief of the Section on Cardiovascular Disease, Cleveland Clinic, Cleveland, Ohio. 102 pp. \$2.50. Springfield Illinois: Charles C Thomas, 1948.

This is a well written and easily read exposition on coronary heart disease in which the pathology, symptomatology, treatment and prognosis of each of the following clinical manifestations of coronary disease are discussed: angina pectoris, acute myocardial infarction, acute coronary failure, paroxysmal cardiac dyspnea (cardiac asthma), heart block and disturbances of cardiac rhythm, and congestive heart failure. In the chapter on paroxysmal cardiac dyspnea, the etiology and pathogenesis of that condition are discussed. The final chapter is devoted to a discussion of risk of anesthesia and of surgical operation. Here the author states that, in general, if anoxia and shock are avoided, patients with coronary heart disease who are able to carry on normal daily activities without experiencing symptoms of myocardial insufficiency, can tolerate general anesthesia and surgery with no more risk than a normal person.

NEWS AND NOTICES

ARMY INSTITUTE OF PATHOLOGY

A direct tribute was recently paid the Army Institute of Pathology and Pathologic Science in general when, on April 26, the United States Senate confirmed the promotion of Colonel Raymond O. Dart, MC, Director of the Army Institute of Pathology, to the rank of Brigadier-General, MC, United States Army. General Dart has spent practically all of his thirty years of service in the field of pathology.

The Journal extends congratulations and cordial good wishes to General Dart.

ARMY MEDICAL MUSEUM

Readers of the Journal will also be pleased to learn that Dr. Ruell A. Sloan, a member of the American Society of Clinical Pathologists, was recently appointed Curator of the Army Medical Museum.

POSTGRADUATE COURSE

A postgraduate course for medical technicians, designed to present the latest advances and technics in clinical pathology, will be given November 22 and 23 at Washington University School of Medicine, St. Louis, Missouri. The course is to consist of lecture, discussion and demonstration periods. Inquiries may be addressed to: Director, Division of Postgraduate Studies, Washington University School of Medicine, 4580 Scott Avenue, St. Louis 10, Missouri.

COURSES IN LABORATORY DIAGNOSIS—U. S. PUBLIC HEALTH SERVICE

The following courses are being offered at the Laboratory Division of the Communicable Disease Center of the U. S. Public Health Service, Atlanta, Georgia:

A two-weeks course in Laboratory Diagnosis of Tuberculosis for laboratory directors, senior staff members and physicians, from October 4 to 15, 1948, and a four-weeks course for laboratory personnel, from November 15 to December 10, 1948.

A six-weeks refresher course for laboratory personnel in the Laboratory Diagnosis of Parasitic Diseases, from October 11 to November 19, 1948.

This training is open to all grades of employed laboratory personnel. Although first consideration will be given to personnel from the laboratories of state and local public health departments, applicants from hospitals and private laboratories will be considered when vacancies occur. Laboratory directors and senior staff members wishing to attend the six-weeks course in the Laboratory Diagnosis of Parasitic Diseases may do so. There is no tuition or laboratory fee, but travel and living expenses must be paid by the individual or his employer.

Applications for the courses should be made by writing to Seward E. Miller, Senior Surgeon, Chief, Laboratory Division, 291 Peachtree Street, Atlanta, Georgia.

SEMINAR IN FORENSIC PATHOLOGY

An intensive course of lectures and demonstrations devoted to the investigation of deaths from obscure or violent causes and how to integrate medical findings with those of the police investigator and the toxicologist will be given by the Department of Legal Medicine, Harvard Medical School, November 8 through November 13, 1948. The attendance will be limited to 25 persons and the fee is \$50.00. Applications for reservations should be forwarded to Dr. Alan R. Moritz, Harvard University Medical School, Department of Legal Medicine, 25 Shattuck Street, Boston 15, Massachusetts.

TECHNICAL SECTION

STUDIES IN SERUM PROTEINS

V. A RAPID PROCEDURE FOR THE ESTIMATION OF TOTAL PROTEIN, TRUE ALBUMIN, TOTAL GLOBULIN, ALPHA GLOBULIN, BETA GLOBULIN AND GAMMA GLOBULIN IN 1.0 ML. OF SERUM*

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AND F. ICHIBA, B.S.

From the Department of Biochemistry, Medical Research Institute, Michael Reese Hospital, Chicago, Illinois

Precipitation methods for the determination of albumin and the three major globulin fractions of serum have been described in previous publications.^{2,3} These technics were shown to give results which agreed well with the results obtained by electrophoretic analysis. The data obtained on human serums by precipitation and by electrophoretic fractionation are summarized in Table 1.

The present report details the procedures by which albumin, total protein and the three major globulin fractions may be rapidly estimated in a small sample of serum. The technic is arranged to use only one standard spectrophotometer curve, and the manipulations involved require approximately twice the time of the standard Howe procedure.

Modified true albumin method. Originally, the albumin filtrate was obtained following sodium sulfite precipitation, by a slow filtration through a double thickness of hardened filter paper. The present modification is considerably faster, since the globulin is removed by centrifugation. The principle employed is that introduced by Kingsley,⁵ who used ether to decrease the density of the globulin precipitated by sodium sulfate. In his method, following ether extraction and brief centrifugation, globulin separates in a compact layer at the bottom of the ether phase above the sodium sulfate phase.

We were unable to apply Kingsley's method directly to the sodium sulfite true albumin procedure. It has been found, however, that addition of a small amount of a suitable surface-active agent (Span 20) to the ether permits centrifugal separation of globulins and does not alter the albumin values obtained. The true albumin values obtained by filtration and after Span-ether treatment and filtration are summarized in Table 2.

Modified gamma globulin method. In both the original procedure and in the present rapid modification, gamma globulin is precipitated by 1.39 M ammonium sulphate at slightly acid pH. An essential feature in all three previously published ammonium sulphate procedures for the isolation and determination of gamma globulin,^{1, 2, 4} is the slow addition of the concentrated precipitant to the serum in order to prevent excessive local concentrations; and, in addition, in all three methods the precipitation is slow and is continued for more than twelve hours.

* Received for publication, May 5, 1948.

It seemed particularly desirable to retain the use of 1.39 M ammonium sulphate since Cohn and co-workers¹ had shown the precipitate to be 90 per cent gamma globulin. This is, for example, a far better yield than is obtained in the ethanol system⁸ where the precipitate first obtained (Fraction II plus III) is only 37 per cent gamma globulin.

It was hoped that rapid mixing of serum and precipitant might be possible if the precipitant concentration were close to the final desired concentration and the dilution were correspondingly great. Accordingly, we decided to work at a dilution of 1 to 25 as it seemed the maximum practicable under routine conditions. Preliminary studies showed, however, that even though a final concentration of 1.39 M in ammonium sulphate was achieved, as the dilution was increased there was a progressive fall in the apparent serum gamma globulin concentration and in the recovery of added gamma globulin.† This proved to result from a decrease in the average ionic strength of the final solution, since it was possible

TABLE 1

AVERAGE DATA OBTAINED BY CHEMICAL FRACTIONATION AND BY ELECTROPHORESIS

SOURCE	NUMBER OF SAMPLES	FRACTIONATION METHOD	VALUES IN GRAMS PER 100 ML.					A/G RATIO
			Albu- min	Total Globu- lin	Alpha Globu- lin	Beta Globu- lin	Gamma Globu- lin	
Cohn and Wolfson ²	4	Chemical	2.40	3.58	1.15	0.94	1.49	0.67
		Electrophoresis	2.48	3.53	1.13	0.92	1.48	0.70
Cohn and Wolfson ³	10	Chemical	2.41	5.24	—	—	—	0.46
		Electrophoresis	2.42	5.14	—	—	—	0.47

to vary the recovery in a more or less linear fashion over a considerable range by adjusting the amount of sodium chloride incorporated into the precipitant.

Figure 1 shows the dependence of the apparent serum gamma globulin concentration and the recovery of added gamma globulin upon the sodium chloride concentration of the precipitant when the ammonium sulfate concentration and the degree of dilution are held constant. Optimum conditions appear to have been achieved at a sodium chloride concentration of 4.0 Gm. per 100 ml. in the precipitant. A decrease in salt concentration to below this value gave poor recoveries of added gamma globulin, and the apparent serum gamma globulin concentrations were too low when compared with electrophoretic checks. Increases in salt concentration led to apparent serum gamma globulin concentrations which were excessive and indicated precipitation of an appreciable portion of the serum beta globulin.

The most gratifying effect of working at greater dilutions and with added sodium chloride is the ease with which the precipitate may be completely spun

† Jager and Nickerson⁴ have apparently made similar observations of the effect of dilution. They state "a number of solubility studies on this (*i.e.*, gamma globulin) fraction reveal reasons why the precipitation from undiluted serum has advantages over the usual procedures in which the serum is diluted greatly prior to precipitation".

down during a short period of centrifugation, a finding which is quite different from the usual behavior of ammonium sulfate precipitates. In our system (using 1.39 M ammonium sulfate) this facilitation of flocculation extends from sodium chloride concentrations of about 0.5 Gm. to about 15.0 Gm. per 100 ml.

Because serum albumin and serum gamma globulin are known to show interaction in certain types of flocculation, it appeared important to discover whether ammonium sulfate precipitation was also affected by albumin concentration. Table 3 summarizes an experiment elucidating this point. The recovery of gamma globulin from synthetic mixtures of albumin and gamma globulin was studied under conditions in which the gamma globulin concentration remained

TABLE 2

SERUM ALBUMIN VALUES OBTAINED FOLLOWING FILTRATION AND FOLLOWING SPAN-ETHER TREATMENT AND CENTRIFUGATION

SAMPLE	VALUES IN GRAMS PER 100 ML.		
	Filtration	Span-Ether	Difference
1	3.5	3.5	0.0
2	3.4	3.4	0.0
3	3.2	3.2	0.0
4	2.9	2.8	-0.1
5	4.1	4.3	0.2
6	3.8	3.8	0.0
7	3.4	3.4	0.0
8*	1.0	1.0	0.0
9*	0.2	0.1	-0.1
10*	0.3	0.4	0.1
Averages.....	2.58	2.59	.

* Nephrotic syndrome, serum lipemic.

constant, while albumin increased from 0.0 Gm. to 8.0 Gm. per 100 ml. The variation noted was insignificant, since all of the amounts recovered fell within the range 92.0 ± 5.0 per cent (1.55 to 1.70 Gm. per 100 ml.). There appeared to be no tendency either toward increased or toward decreased precipitation associated with a change in albumin concentration. This finding implies that little or no mechanical occlusion of albumin occurs during the course of precipitation and suggests that washing of the precipitate, advised by Jager and Nickerson,⁴ is unnecessary in our procedure. This has been confirmed for native serums since gamma globulin values of washed samples were found to average 98 per cent of the values in unwashed samples.

It is apparent from these data that precipitation of gamma globulin by ammonium sulfate does not share with Kunkel's copper sulfate gamma globulin determination the defect of being dependent upon albumin concentrations.⁶ It is interesting that the cephalin-cholesterol flocculation reaction resembles Kunkel's reaction more closely in that it is inhibited by normal serum albumin,

(but not by serum albumin from patients with hepatitis) while it is accelerated by serum gamma globulin from any source.⁷

Our recoveries of gamma globulin added to serum or from artificial mixtures of albumin and gamma globulin have averaged about 85 per cent with an extreme range of 78 per cent to 92 per cent in various experiments. It is our impression that the best recoveries may be obtained only by following scrupulously the directions given in the third, fourth and fifth steps of the procedure given below. Our recoveries compare quite favorably with the 90 per cent recovery which was obtained by Cohn and his co-workers¹ under optimum conditions of maximal control and with the 73 per cent to 83 per cent recovery reported by Jager and Nickerson⁴ under conditions more comparable to ours. Since the presence or absence of other proteins does not appear to affect the recoveries appreciably, it

TABLE 3

THE EXTENT OF GAMMA GLOBULIN PRECIPITATION IS NOT ALTERED BY CHANGES IN THE CONCENTRATION OF SERUM ALBUMIN

SAMPLE*	VALUES IN GRAMS PER 100 ML.			PER CENT RECOVERY
	Albumin	Gamma Globulin, Prepared	Gamma Globulin, Recovered	
A	0.00	1.75	1.55	88.5
B	1.00	1.75	1.65	94.4
C	2.00	1.75	1.55	88.5
D	4.00	1.75	1.60	91.5
E	8.00	1.75	1.70	97.1
Averages	—	1.75	1.61	92.0

* The samples were prepared from suitable amounts of salt-free human albumin (Cutter Laboratories) and human immune serum globulin (Cutter Laboratories) using 0.85 per cent saline as a diluent.

may be assumed, tentatively, that the average recovery of 85 per cent, obtained when gamma globulin is added to serum, applies as well to the precipitation of gamma globulin from native serums. Thus, of the 1.20 Gm. of "gamma globulin" which is precipitated from 100 ml. of average pooled normal serum, it is likely that 1.02 Gm. is actually gamma globulin. The remaining 0.18 Gm. is probably chiefly beta globulin and alpha globulin, as in Jager and Nickerson's precipitates, but this amount is only 8.8 per cent of the average serum alpha and beta globulin. The selectivity of the gamma globulin fractionation is, therefore, of the general order found in most routine biochemical separations and is quite satisfactory for clinical use and for most investigative purposes.

Detailed working instructions for the various fractionation procedures follow. Results obtained in certain clinical problems in which these procedures have been employed on a large scale will be reported elsewhere.^{10, 11}

PROCEDURE

Reagents

23.0 per cent sodium sulfate solution. Dissolve exactly 23.0 grams of anhydrous sodium sulfate in distilled water at 37 C. Make up to 100 ml. with distilled water and store in an incubator at 37 C.

28.0 per cent sodium sulfite solution. Dissolve exactly 28.0 grams of anhydrous sodium

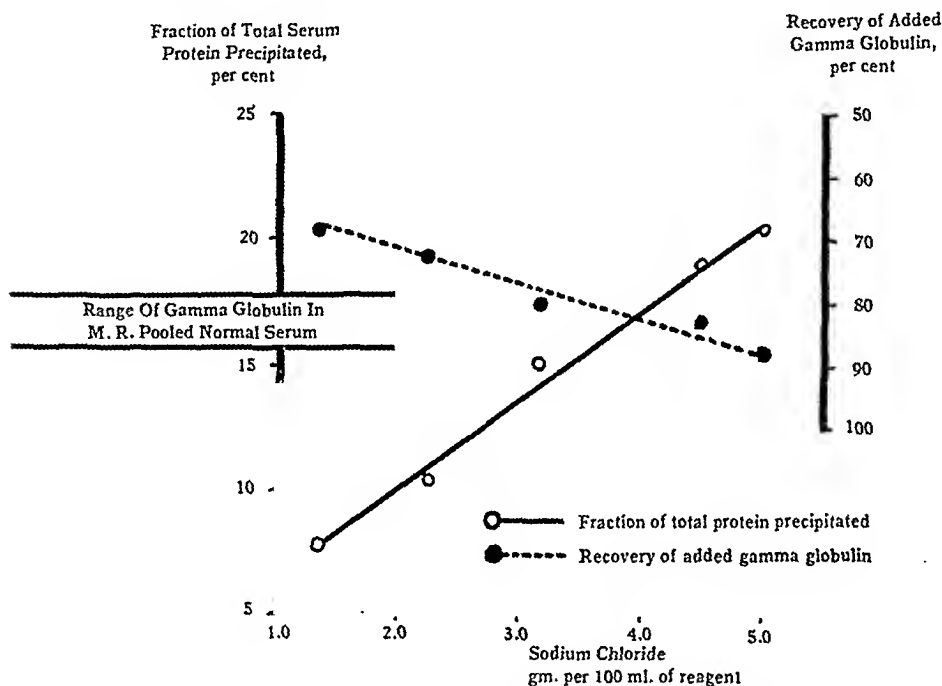


FIG. 1. FACTORS DICTATING THE CHOICE OF THE GAMMA GLOBULIN REAGENT EMPLOYED

Reagent samples were prepared by placing 19.3 Gm. of ammonium sulfate in a graduated diluting cylinder, adding the requisite amount of sodium chloride and diluting to 100 ml. with distilled water. In recovery experiments, commercial "immune serum globulin" was employed. According to Mulford,⁸ such material contains 85 per cent to 95 per cent gamma globulin. For purposes of calculation, we assumed a gamma globulin content of 90 per cent.

The concentration of sodium chloride finally chosen, 4.0 Gm. per 100 ml., was that which appeared to give a maximum recovery of added gamma globulin consistent with a minimum precipitation of beta globulin from the serum.

sulfite in distilled water at 28 C. It is difficult to make the salt soluble, but it can be dissolved with sufficient shaking. Make up to 100 ml. with distilled water and store at room temperature.

Saline ammonium sulfate solution. In a one liter volumetric flask, dissolve 193 grams of ammonium sulfate in about 500 ml. of distilled water. Add 40 grams of sodium chloride, dissolve and make up to one liter with distilled water. Store at room temperature.

Biuret reagent, Weichselbaum.⁹ Prepare an accurately titrated 0.2 N NaOH solution. Dissolve 90 grams of Rochelle salt in about 400 ml. of the 0.2 N NaOH solution. Following solution, add 10 grams of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$. When this copper sulfate has entirely dissolved, add 10 grams of potassium iodide and make up to two liters with 0.2 N NaOH. Store in a rubber-stoppered waxed glass bottle.

Span-ether reagent. Mix 1 ml. of Span 20 (Atlas Powder Company, Wilmington, Delaware) with 99 ml. of ether, U.S.P. Filter, through a moderately fast paper into a 100 ml. diluting cylinder and make up to 100 ml. with ether. Store in a tightly corked bottle. Ether, U.S.P.

Standardization

1. The standard protein solution may be either of the following:
 - a. A sample of pooled normal human serum, the protein content of which has been determined accurately by the Kjeldahl method.
 - b. Commercial human albumin solution. Although expensive, this has the advantage of having a protein concentration of about 25 Gm. per 100 ml. and permits standards to be prepared with protein concentrations well in excess of normal serum. As in the case of pooled normal serum, the exact protein concentration must be determined by Kjeldahl analysis.
2. Using normal saline as the diluent, prepare standards with protein concentrations of about 1.0, 2.0, 4.0, 6.0 and 8.0 Gm. per 100 ml. Determine the protein concentration of each dilute standard by the total protein procedure given below. Construct the standard curve from the data obtained.

Determinations

1. *Serum total protein.*
 - a. Pipet 0.2 ml. of serum into a 10 ml. graduated mixing cylinder and dilute to 5 ml. with distilled water. Mix well by inversion.
 - b. Transfer 3.0 ml. to a cuvet, add 3.0 ml. of biuret reagent and mix well by shaking.
 - c. Prepare the blank with 3.0 ml. of distilled water and 3.0 ml. of biuret reagent. Save this blank for use in the determination of albumin plus alpha globulin (Step 2f) and in the determination of gamma globulin (Step 8g).
 - d. Let the solutions stand at least 30 minutes. (Since the color is quite stable, readings may be deferred for as much as 24 hours.) Read on the photoelectric colorimeter or spectrophotometer at a wavelength of 540 millimicrons. (The Evelyn photoelectric colorimeter was used in our studies.)
2. *Serum albumin plus alpha globulin.*
 - a. Place 2.3 ml. of 23.0 per cent sodium sulfate solution in a test tube. This solution is best not pipetted, since it has a definite tendency to crystallize, particularly on cold pipets. We have found it convenient to use small calibrated tubes marked at 2.3 ml. and to fill these rapidly from a large buret, preparing enough at one time for a day's determinations.
 - b. Pipet in 0.2 ml. of serum and mix thoroughly by inversion.
 - c. Add approximately 1 ml. of ether and shake vigorously for 30 seconds. Centrifuge for 5 to 10 minutes at 1500 to 2000 r.p.m.
 - d. After centrifugation, carefully insert a pipet through the ether layer and beneath the packed globulin, slanting the tube to separate the precipitate from the wall of the tube.
 - e. Withdraw 1.5 ml. of clear centrifugate and transfer to a cuvet. Add 1.5 ml. of distilled water and 3.0 ml. of biuret reagent. Mix well by shaking.
 - f. The blank is that prepared in Step 1c.
 - g. After standing at least 30 minutes, read on the photoelectric colorimeter at a wavelength of 540 millimicrons.
3. *Serum albumin.*
 - a. Place 4.8 ml. of 28 per cent sodium sulfite solution in a test tube. It is suggested that a procedure similar to that advised in Step 3a be used in preparing these tubes.
 - b. Pipet in 0.2 ml. of serum and mix thoroughly by inversion.

- c. Add about 1 ml. of Span-ether reagent and shake vigorously for 30 seconds. Centrifuge for 5 to 10 minutes at 1500 to 2000 r.p.m.
- d. After centrifugation, carefully insinuate a pipet through the Span-ether layer and beneath the packed globulin, slanting the tube to separate the precipitate from the wall.
- e. Withdraw 3.0 ml. of the clear centrifugate and transfer to a cuvet. Add 3.0 ml. of biuret reagent and mix well by shaking.
- f. Prepare the blank with 3.0 ml. of sodium sulfite solution and 3.0 ml. of biuret reagent.
- g. After standing at least 30 minutes, read on the photoelectric colorimeter at a wavelength of 540 millimicrons.
4. *Serum total globulin.* Subtract the value for serum albumin obtained in Step 3g from the value for serum total protein obtained in Step 1d.
5. *True A/G ratio.* Divide the value for serum albumin obtained in Step 3g by the value of serum total globulin obtained in Step 4.
6. *Serum alpha globulin.* Subtract the value for serum albumin obtained in Step 3g from the value for serum albumin plus alpha globulin obtained in Step 2 g.
7. *Serum beta plus gamma globulin.* Subtract the value for serum albumin plus alpha globulin obtained in Step 2g from the value for serum total protein obtained in Step 1d.
8. *Serum gamma globulin.*
 - a. Pipet 9.6 ml. of saline ammonium sulfate into a sturdy 15 ml. thick walled glass or plastic test tube. Layer 0.4 ml. of serum on top of the saline ammonium sulfate. Mix the two components by careful, slow, repeated inversion. Continue mixing until, within a minute or two, the gradually developing visible turbidity has reached an apparent maximum.
 - b. Remove 1.0 ml. of the mixture with a pipet and discard.
 - c. *Cork the tube securely* and centrifuge at 2250 to 2750 r.p.m. for 30 minutes. If, at the end of this time, the supernatant is found to be somewhat turbid, cool the tube for a few minutes under the cold water tap and centrifuge again. Accurate results are obtained only when the supernatant is crystal clear.
 - d. Being extremely careful not to disturb the precipitate, gently turn the uncorked tube on its side and permit the supernatant to run off. No attempt should be made to insure complete removal of the supernatant at this time.
 - e. Return the uncorked tubes to the centrifuge and spin at 2250 to 2750 r.p.m. for five minutes. Now slowly invert the tubes and let them stand in this position on a layer or two of paper toweling or filter paper for a few minutes.
 - f. Add 3.0 ml. of biuret reagent and 3.0 ml. of distilled water to the tube and shake briskly for 30 seconds. Let stand 15 minutes, centrifuge down any slight residual turbidity remaining, and decant the supernatant into a cuvet.
 - g. Prepare the blank with 3.0 ml. of biuret reagent and 3.0 ml. of water. The blank prepared in Step 1c may be saved and used.
 - h. After it has stood at least 30 minutes, read on the photoelectric colorimeter at a wavelength of 540 millimicrons. Divide the protein value obtained by 3 to obtain the serum gamma globulin concentration.
9. *Serum beta globulin.* Subtract the value for serum gamma globulin obtained in Step 8h from the value for serum beta plus gamma globulin obtained in Step 7.

Normal Values

Values obtained on four samples of pooled normal human serum are given below. Each pool contained serums from between 30 and 150 persons. The chemical fractionation data on the first two samples were checked by electrophoretic fractionation. The data are averages of several determinations on each sample.

Values in Grams per 100 ml.

SAMPLE	TOTAL PROTEIN	ALBUMIN	TOTAL GLOBULIN	ALPHA GLOBULIN	BETA GLOBULIN	GAMMA GLOBULIN	A/G RATIO
1	7.20	3.85	3.35	1.29	0.77	1.29	1.15
2	6.95	3.78	3.17	1.46	0.66	1.05	1.19
3	6.70	3.60	3.10	0.70	1.25	1.15	1.16
4	7.20	3.85	3.35	0.95	1.05	1.35	1.15
Averages	7.01	3.77	3.24	1.10	0.94	1.20	1.16

SUMMARY

Simple procedures have been described for the estimation of total protein, true albumin, total globulin, alpha globulin, beta globulin, and gamma globulin in a 1.0 ml. sample of serum. The method is rapid and permits the simultaneous handling of large numbers of samples.

Acknowledgment. We wish to thank the Atlas Powder Company of Wilmington, Delaware for its cooperation in supplying us with liberal samples of a large number of surface-active agents of the Span and Tween series for trial. Pooled normal human serum was obtained through the kind assistance of the Samuel Deutsch Serum Center, Michael Reese Research Foundation.

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AN IMPROVED ANTIGEN FOR THE KOLMER COMPLEMENT-FIXATION TEST FOR SYPHILIS*

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The original Kolmer antigen, a cholesterolized and lecithinized alcoholic extract of beef heart, was described in 1922. It was prepared by extracting beef heart powder with ether, followed by primary and secondary extractions of the tissue residue with ethyl alcohol, re-enforcement of the secondary alcoholic extract with acetone-insoluble lipoids recovered from the preliminary ether and primary alcoholic extracts, and sensitization of the antigen with 0.2 per cent cholesterol.⁵ In 1935 an alternate method was described in which the antigen was prepared in the same manner except that beef heart powder was first extracted with acetone instead of with ether as in the original method.⁶ This change in technic was based upon the observation that preliminary extraction of beef heart powder with acetone also reduced the anticomplementary and other nonspecific properties of the antigen.

Further confirmation of the advisability of preliminary removal of the acetone-soluble lipoids has been supplied by the fact that beef heart powder, from which cardiolipin and lecithin are prepared, is first liberally extracted with acetone.¹⁵ Furthermore, in November 1946, we were informed by Dr. George W. Cox that Mr. K. C. Knolle, serologist in the Bureau of Laboratories of the Texas State Department of Health, had observed that the nonspecific effects of Kolmer antigen, prepared according to the alternate method, were still further reduced by washing the acetone-extracted powder on the filter three times with acetone before the residue was extracted with ethyl alcohol.

We have been able to confirm these observations although, since acetone-soluble lipoids are also antigenic,¹³ their removal from beef heart powder before extraction with ethyl alcohol sometimes reduces the sensitivity of antigens. Furthermore, we are now convinced that it is equally important also to remove the ether-soluble lipoids, as in the original Kolmer method, in view of their well known anticomplementary and hemolytic properties.^{13, 14} Indeed, it would appear that these lipoids occurring in antigens may be even more responsible for prezone and nonspecific Kolmer complement-fixation reactions than the acetone-soluble lipoids. Under the circumstances we now prepare Kolmer antigen by first extracting beef heart powder with acetone followed by extraction with ether, before the tissue residue is extracted with ethyl alcohol. The latter is then sensitized by adding 0.4 instead of 0.2 per cent cholesterol to restore the loss of sensitivity due to the removal of the acetone-soluble and ether-soluble lipoids.

* Received for publication, April 9, 1948.

IMPROVED KOLMER ANTIGEN

The details of preparation are as follows:

1. Weigh out 30 Gm. of beef heart powder. (Baeto beef heart, prepared by the Difco Laboratories, is recommended.)
2. Transfer to a flask fitted with a glass stopper or tin foil-covered cork.
3. Add 100 cc. of chemically pure acetone.
4. Stopper tightly and mix thoroughly.
5. Keep at room temperature for two days, shaking briefly each day.
6. Filter through fat-free paper, with slight squeezing of the tissue, and discard the filtrate.
7. Transfer the residue to the flask, add 100 cc. of chemically pure acetone, stopper tightly, mix thoroughly and keep at room temperature for two days, shaking briefly each day.
8. Filter through fat-free paper, with slight squeezing of the tissue, and discard the filtrate.
9. Dry the residue in the air until free of acetone, and return it to a clean flask.
10. Add 200 cc. of ether, stopper tightly, mix thoroughly, and keep at room temperature for three days, shaking briefly each day.
11. Filter through fat-free paper with slight squeezing of the tissue.
12. Dry the residue in the air until free of ether, and return it to a clean flask.
13. Add 100 cc. of chemically pure absolute ethyl alcohol.
14. Stopper flask tightly, and mix thoroughly.
15. Store at room temperature for five days, shaking each day.
16. Filter through fat-free paper, with slight squeezing of the tissue.
17. Measure the filtrate, and filter sufficient absolute ethyl alcohol through the tissue to make 100 cc.
18. Weigh out 0.4 Gm. of cholesterol, and dissolve in 10 cc. of ether.
19. Add to the 100 cc. of alcoholic filtrate.
20. Shake thoroughly, and place in a water bath at 55 C. for one hour to aid solution.
21. Allow to stand at room temperature for two days, shaking briefly each day.
22. Filter through fat-free paper.
23. Store the antigen at room temperature in the dark in a tightly stoppered bottle. Do not disturb any sediment which may be present.

IMPROVED KOLMER ANTIGEN (RE-ENFORCED)

It will be observed that we do not include re-enforcement of the antigen with acetone-insoluble lipoids, as originally described,⁵ since the percentage of cholesterol has been increased from 0.2 to 0.4 per cent. While the addition of these lipoids increases the sensitivity of antigens as originally prepared,^{5, 12} so far we have observed that their addition to the improved antigen may or may not increase sensitivity. Furthermore, re-enforcement with acetone-insoluble lipoids may tend to yield prezone and nonspecific reactions, especially in spinal fluid tests, although we have not observed these effects in our laboratory, up to the present time at least. The technic of preparation is as follows:

1. Steps 1 through 17 as above.
2. Save the ether extract used in the preparation of the antigen in steps 10 and 11.
3. Place in an evaporating dish and concentrate to about one-fifth volume.
4. Add 3 to 6 volumes of acetone.
5. Mix and set aside overnight.
6. Remove supernatant acetone, and store the residue of acetone-insoluble lipoids in the refrigerator.
7. Dissolve 0.4 Gm. of cholesterol and the acetone-insoluble lipoids in 10 cc. of ether.
8. Add to the 100 cc. of alcoholic filtrate; stopper tightly, and shake thoroughly.
9. Place in a water bath at 55 C. for one hour to aid solution.
10. Allow to stand at room temperature for two days, shaking briefly each day.
11. Filter through fat-free paper.
12. Store at room temperature in the dark. Do not disturb any sediment which may be present.

PROPERTIES OF THE IMPROVED ANTIGEN

With improved antigen prepared without re-enforcement by acetone-insoluble lipoids, we have found it unnecessary to use pretested guinea pig complement in the Kolmer complement-fixation test. In 1939, Giordano and Carlson³ observed that the serums of some guinea pigs apparently contain a substance which fixes or inactivates complement in the presence of antigen at refrigerator temperature, with the suggestion that each guinea pig serum employed as complement be subjected to preliminary testing before use in the Kolmer complement-fixation test. These observations were confirmed by Kolmer and Lynch⁹ who thought this phenomenon was responsible not only for false-positive reactions, but for prezone reactions as well, sometimes observed in complement-fixation tests and especially with spinal fluids. The results of an investigation by Harris,⁴ however, have indicated that these nonspecific reactions were not due to the presence of a substance in guinea pig complement serums but, apparently, to a substance or substances occurring in some antigens, which we now surmise may have been acetone and/or ether-soluble lipoids. Be that as it may, we have found it unnecessary to use pretested complement with the improved antigen. With this antigen, we have also found it unnecessary to use egg albumin for the prevention of nonspecific and prezone reactions, especially in spinal fluid examinations, as advised by Boerner and Lukens.^{1, 10} Furthermore, prezone reactions with human serums, in the Kolmer quantitative complement-fixation test, have been but rarely observed and false-positive reactions with normal spinal fluids in the Kolmer simplified and quantitative tests have been of rare occurrence. In these respects Improved Kolmer Antigen has behaved in the same manner as cardiolipin-lecithin-cholesterol antigen (0.03-0.05-0.6) in Kolmer complement-fixation tests in our laboratory.

As elsewhere reported,¹¹ we have found this improved antigen of about the same sensitivity as cardiolipin-lecithin-cholesterol antigen (0.3-0.05-0.6) in the Kolmer simplified complement-fixation test for syphilis insofar as the percentages of positive and doubtful reactions are concerned; in the quantitative Kolmer complement-fixation test, however, cardiolipin antigen has been found sometimes to yield somewhat more sensitive reactions. Both antigens have shown practically 100 per cent negative reactions with the serums and spinal fluids of presumably normal nonsyphilitic individuals. In this connection it may be stated that Kolmer complement-fixation tests in our laboratory have given 100 per cent negative reactions with the serums of 1116 presumably normal nonsyphilitic donors in the 1935-43 National Serologic Surveys and the 1941 Washington serology conference⁷ although four false-positive and 2 doubtful reactions were reported by us in the tests with the serums of 575 donors in the 1944-47 National Serologic Surveys. In the 1948 National Serologic Survey all quantitative Kolmer complement-fixation tests were conducted in our laboratory with our improved antigen and cardiolipin-lecithin-cholesterol antigen (0.03-0.05-0.6); the announcement of specificity and sensitivity ratings with these two antigens is now being awaited. In this connection it may be stated, however, that both

antigens have given about the same percentage of biologic false-positive Kolmer complement-fixation reactions with the serums of 42 normal rabbits, although cardiolipin-lecithin-cholesterol antigen gave stronger reactions in about 10 per cent. With both antigens, therefore, a special technic must be employed in Kolmer complement-fixation tests with rabbit serums to avoid false-positive or nonspecific reactions.⁸

Insofar as the stability of this improved antigen is concerned, it is too soon for us to express an opinion, but experience with Kolmer antigens during the past twenty-six years has shown quite conclusively that there are usually no detectable changes in antigenicity and anticomplementary properties over a period of one to three years when antigens are kept tightly stoppered at room temperature in the dark.

TABLE 1
COMPARATIVE ANTIGENIC SENSITIVITY OF DIFFERENT IMPROVED KOLMER ANTIGENS

PARTS SERUM POS. + NEG.	ANTIGENS*			
	No. 1	No. 2	No. 3	No. 4
Undiluted	4 4 4 4 1	4 4 4 4 2	4 4 4 4 -	4 4 4 4 2
1 + 4	4 4 4 4 -	4 4 4 4 -	4 4 4 3 -	4 4 4 4 -
1 + 9	4 4 2 1 -	4 4 2 1 -	4 4 1 - -	4 4 3 1 -
1 + 19	3 2 1 - -	3 2 1 - -	3 2 - - -	3 2 1 - -
1 + 39	- - - - -	- - - - -	- - - - -	- - - - -
1 + 79	- - - - -	- - - - -	- - - - -	- - - - -

* Antigens No. 1, 2 and 3 represent improved Kolmer antigens, antigen No. 4, improved Kolmer antigen (re-enforced).

One distinct advantage of cardiolipin is stated to be its chemical reproducibility. More difficulty has been experienced in the chemical reproducibility of lecithin, presumably due to fatty acids, which is employed along with cholesterol in the sensitization of cardiolipin for antigen in both complement-fixation and flocculation tests.

We have made no attempt to isolate or identify the antigenic lipoids in Kolmer antigen except to state that Brown and Kolmer² observed, in 1941, that antigenic activity cannot be due to either lecithin or cephalin, but to an unknown substance which, possibly, could have been cardiolipin which was subsequently isolated and identified by Pangborn.¹⁵ At all events it is apparent that the method of preparation of our improved antigen alone does not insure antigens of strictly reproducible qualities. But, comparative Kolmer quantitative complement-fixation tests with different improved Kolmer antigens, prepared on different occasions from different lots of beef heart powder, have yielded closely similar results, as shown in Tables 1 and 2. Table 1 shows the results of comparative tests with three improved Kolmer antigens and one improved Kolmer antigen (re-enforced) tested with syphilitic serum diluted with normal serum. It will be observed that the results are closely similar. Similar results were observed when

10 individual syphilitic serums were tested simultaneously with three different improved antigens (Table 2). It is likely that similar tests with different lots of cardiolipin-lecithin-cholesterol antigens may give more strictly comparative results; but, we have had no opportunity to make such comparative examinations, as all of our work with cardiolipin up to the present time has been conducted with antigens prepared of the same stock alcoholic solutions of cardiolipin and lecithin. In routine simplified Kolmer complement-fixation tests with syphilitic serums and spinal fluids the results have been practically identical with different improved antigens insofar as positive and doubtful reactions are concerned. In routine Kolmer quantitative complement-fixation tests, however, some antigens have been found more sensitive than others. Since we have been working

TABLE 2

COMPARATIVE ANTIGENIC SENSITIVITY OF DIFFERENT IMPROVED KOLMER ANTIGENS

SYPHILITIC SERUMS	ANTIGENS*		
	No. 1	No. 2	No. 3
1	4 4 4 4 -	4 4 4 4 -	4 4 4 3 -
2	4 4 4 4 -	4 4 4 4 -	4 4 4 4 -
3	4 4 4 2 -	4 4 4 1 -	4 4 3 - -
4	4 4 4 4 -	4 4 4 3 -	4 4 4 1 -
5	4 4 4 4 4	4 4 4 4 4	4 4 4 4 4
6	4 4 4 4 1	4 4 4 4 4	4 4 4 4 2
7	4 4 1 - -	4 4 2 - -	4 4 - - -
8	4 2 - - -	4 3 - - -	4 2 - - -
9	2 - - - -	2 - - - -	1 - - - -
10	1 - - - -	1 - - - -	± - - - -

* Improved Kolmer antigens were used in doses of 0.5 cc. of 1:600 dilution.

with but one cardiolipin-lecithin-cholesterol antigen up to the present time, we are unable to state how different lots compare in uniformity of reactions in the Kolmer simplified and quantitative tests.

SUMMARY

1. An improved method is described for the preparation of Kolmer antigen.
2. This improved antigen has been found closely similar to cardiolipin-lecithin-cholesterol antigen (0.03-0.05-0.6) in antigenic sensitivity in the Kolmer simplified and quantitative complement tests for syphilis.
3. Both antigens have been found of equal specificity in Kolmer complement-fixation tests with the serums and spinal fluids of presumably normal nonsyphilitic persons.
4. Until the value of cardiolipin-lecithin-cholesterol antigen in the Kolmer complement-fixation tests is more fully established in relation to the diagnosis and treatment of syphilis, it appears advisable to use it side by side with the improved Kolmer antigen herein described.

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EFFECT OF HUMAN CEREBROSPINAL FLUID ON THE DILUTION BIOASSAY OF PENICILLINS G, X AND K*

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Numerous workers have been unable to detect penicillin in the cerebrospinal fluid of man after extrathecal administration of the drug in the usual therapeutic doses. Significant concentrations may, however, be demonstrated with some degree of regularity in the presence of some forms of bacterial meningeal irritation,⁵ or when massive intravenous dosages are given.¹ (See Dumoff-Stanley and her co-workers,² Schwemlein and others⁶ for detailed discussion.) It is the purpose of this preliminary report to point out that human cerebrospinal fluid exerts an inhibitory effect on the bactericidal activities of penicillins G, X, and K *in vitro* as determined by a serial dilution bioassay method. This effect is comparable in some ways to that exerted by human serum,^{3, 4} but differs in several important respects. Whatever the mechanism involved, the inability to demonstrate even traces of penicillin in normal cerebrospinal fluid after its intramuscular administration in average therapeutic dosage may be related to the inhibitory effect of spinal fluid on the bactericidal activity of the drug.

METHOD AND MATERIALS

Individual and pooled cerebrospinal fluid specimens obtained from normal persons and syphilitic patients were used in this study. The syphilitic patients were either untreated, or had received penicillin therapy for neurosyphilis, but in no case more recently than three weeks previously. Total protein determinations were carried out by a turbidimetric method (Klett-Summerson) on several pooled specimens, and in no case did this value exceed 50 mg. per 100 ml.

The principle of the method here employed has been described in a similar study on individual and pooled human serums.⁴ In the control assay, containing no cerebrospinal fluid, varying amounts of a penicillin dilution in broth (0.8, 0.72, 0.6, 0.48, 0.4 ml., etc.), were adjusted to a total volume of 0.8 ml. with broth. A 4 per cent broth suspension of defibrinated human group O blood was inoculated with the C-203 strain of *Streptococcus pyogenes*; 0.5 ml. of this mixture was then added to each tube. The blood served as a hemolytic indicator of growth of the streptococcus. The end point was the tube containing the smallest amount of penicillin which had completely inhibited hemolysis after six hours' incubation at 37 C. followed by ten to twelve hours' incubation at room temperature.

In order to determine the effect of cerebrospinal fluid on the results of this

* The experimental portion of the work was done in the U.S.P.H.S. Laboratory of Experimental Therapeutics, Dr. Harry Eagle, Director. Received for publication, June 5, 1948.

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assay, dilutions of penicillin in 96, 48, 24, 12 and 6 per cent spinal fluid were similarly distributed, and the volume adjusted to 0.8 ml. with the corresponding spinal fluid-broth diluent (e.g., one volume of 1:1,000,000 penicillin in broth was diluted with 24 volumes of 50 per cent fluid to give a 1:25,000,000 dilution in 48 per cent cerebrospinal fluid. This was distributed and the volumes adjusted to 0.8 ml. by the addition of 48 per cent spinal fluid in broth). In this manner a

TABLE 1
INHIBITORY EFFECT OF HUMAN CEREBROSPINAL FLUID ON THE *in vitro* ACTIVITIES OF
PENICILLINS G, X AND K

PENI- CILLIN SPECIES	PERCENTAGE OF SPINAL FLUID IN ASSAY		RELATIVE ACTIVITY OF PENICILLIN IN SPINAL FLUID SPECIMEN NUMBER, (BASED ON ACTIVITY OF 100 IN BROTH CONTROL)											RELATIVE ACTIVITY	
	Before Addi- tion of Red Blood Cell Sus- pen- sion	After Addi- tion of Red Blood Cell Sus- pen- sion												Mean	Me- dian
			1	2	3	4	5	6	7	8	9	10	11		
G	96	59	56	56	50	50*	60	55	55	50	55	55	45	53.4	55.0
	48	29.5	75	75	67	60	73	67	61	61	67	61	60	66.1	67.0
	24	14.8	75	75	83.5	75	86	79	73	67	76	76	75	76.5	75.0
	12	7.4	100	90	83.5	90	100	92	92	83	100	92	75	90.5	92.0
	6	3.7	100	100	83.5	95	100	92	100	91	100	92	90	94.0	95.0
X	96	59	56	60	50	50	50	50	61	55.5	55.5	62	60	55.5	55.5
	48	29.5	60	69	60	55.5	55.5	55.5	71.5	71.5	71.5	80	80	66.4	69.0
	24	14.8	90	100	80	80	80	73	83.5	83.5	83.5	80	80	83.0	80.0
	12	7.4	100	100	80	86	86	80	100	100	91	80	80	89.4	86.0
	6	3.7	100	100	86	100	100	100	100	100	100	100	100	98.7	100.0
K	96	59	53	53	62.5*	62.5*	62.5*	62.5*	54.5	50	51.5	46*	46*	55.2	51.5
	48	29.5	68	63	91	83	83	83	63	75	63	61	52.5	71.4	68.0
	24	14.8	79	—	100	100	100	83	75	75	75	73.5	73.5	83.4	77.0
	12	7.4	95	—	100	100	100	100	80	80	80	92	78	90.5	93.5
	6	3.7	100	100	100	100	100	100	80	80	80	100	100	94.5	100.0

* Determinations with spinal fluid from nonsyphilitic subjects.

constant percentage of cerebrospinal fluid was maintained in each tube of the assay. Finally, as with the control assay, 0.5 ml. of red blood cell suspension was added to each tube; this reduced the concentration of spinal fluid by $\frac{0.8}{0.8 + 0.5}$. Thus, an initial concentration of 96 per cent fell to 59 per cent, 48 per cent to 29.5, etc., as indicated in Tables 1 and 2. The amount of penicillin necessary to inhibit hemolysis in the presence of varying concentrations of spinal fluid, compared to the amount necessary in its absence, provided a direct measure of the degree to which the activity of the drug had been inhibited by a particular concentration of cerebrospinal fluid.

RESULTS

The results of 11 determinations with each penicillin studied (G, X and K) are presented in Table 1. Significant differences were not found between individual and pooled specimens, nor did fluids obtained from normal subjects differ demonstrably in their inhibitory effect from those taken from patients with untreated syphilis or penicillin-treated neurosyphilis. The results were, therefore, combined, and the resulting average curves are shown in Figure 1.

Penicillins G, X and K proved identical in their susceptibility to the inhibitory effect of spinal fluid. Further, the inhibitory effect resembled that of human serum on penicillin G and X. The effect of unheated human serum on penicillin K,

TABLE 2

EFFECT OF HUMAN CEREBROSPINAL FLUID ON THE BIOASSAY OF PENICILLINS G, X AND K:
INTERPOLATED CORRECTIVE FACTORS
(After Table 1, Figure 1)

AMOUNT OF SPINAL FLUID (IN TOTAL VOLUME OF 0.8 ML.) IN INDICATOR TUBE OF ASSAY ¹	PERCENTAGE OF SPINAL FLUID		CORRECTIVE FACTORS (MEANS) FOR PENICILLINS G, X AND K ²
	Before Addition of Red Cell Suspension	After Addition of Red Cell Suspension	
0.8	whole fluid = 100	61.5	1.8
0.7	1:1.1 = 87.5	54	1.7
0.6	1:1.3 = 75	46	1.65
0.5	1:1.6 = 62.5	38.5	1.6
0.4	1.2 = 50	31	1.5
0.3	1:2.5 = 37.5	23	1.4
0.2	1.3 = 25	15.5	1.3
0.15	1.4 = 19	11.5	1.2
0.10	1.8 = 10	6	1.1
<0.10	1:16+ = <7	<5	1.0

¹ Smallest amount of penicillin which, in a total volume of 0.8 ml., inhibited completely hemolysis of human red cells by the C-203 strain of *Streptococcus pyogenes*.

² Factors by which the apparent concentration of penicillin in a particular concentration of spinal fluid must be multiplied to compensate for inhibitory effect of fluid on the assay.

however, was much greater than that exerted by cerebrospinal fluid. Thus, in the presence of 59 per cent human serum, the apparent activity of penicillin K was only 6 per cent, as contrasted to 36 per cent for G and 40 per cent for penicillin X; while in the presence of 59 per cent spinal fluid, the values for the activities of the three penicillins were 55, 53 and 56 per cent, respectively.

Preliminary studies (not cited in the tables) have shown that this inhibitory effect was not diminished appreciably by heating at 100 C. for thirty minutes, followed by Seitz filtration of the spinal fluid. Changes in hydrogen ion concentration, within the range of pH 7.3 to 8.4, did not alter the effect. As will be described in a following paper, when various concentrations of the penicillins were incubated in 96 per cent cerebrospinal fluid at 37 C., there was progressive loss of penicillin activity; and the degree of inactivation was unaffected by the initial concentration of the drug over a 500-fold range (1:10,000-1:5,000,000).

DISCUSSION

Although the inhibitory effect of human serum on the bioassay of penicillin has been ascribed, at least in large part, to the binding effect of the plasma proteins (particularly of the albumin fraction),⁷ such a mechanism does not explain the similar property of spinal fluid. For penicillins G and X, the inhibitory effect of cerebrospinal fluid was only 15 to 20 per cent less than that of serum, while the total protein contents differed by a factor of 100- to 300-fold. Further,

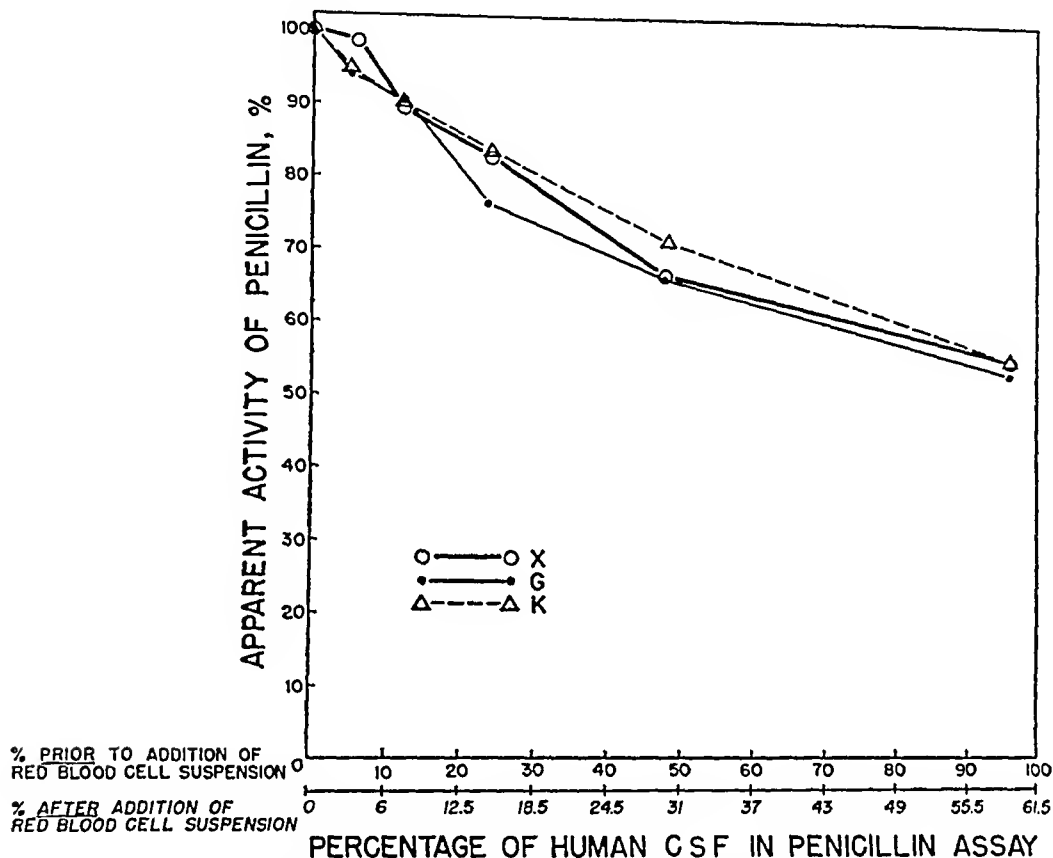


FIG. 1. Inhibitory effect of human cerebrospinal fluid on *in vitro* activities of penicillins G, X, and K. The abscissas are percentages of spinal fluid present in the penicillin assay, before and after addition of red blood cell suspension (hemolytic indicator). The ordinates are the mean apparent activities of the penicillins.

heat-coagulation of the protein and its removal by means of Seitz filtration did not modify appreciably the inhibitory effect. Finally, the fact that penicillin, incubated in cerebrospinal fluid, progressively lost activity, suggested that there might be actual *destruction* of penicillin by some, as yet unidentified, mechanism similar to that observed in the case of serum.³ Further studies are in progress and will be reported subsequently.

From the practical standpoint, it becomes feasible to correct the results of bioassays on human cerebrospinal fluid for the inhibitory effect exerted by the fluid, provided only that we know the concentration of spinal fluid in the indicator tube of the assay. In Table 2 are given corrective factors by which the ap-

parent penicillin content should be multiplied in order to compensate for this inhibitory effect. Since the curves for penicillins G, X and K did not differ significantly (Fig. 1), the same corrective factors apply to all three species. The corrections are significant only when the final concentration of spinal fluid (after the addition of the red blood cell suspension), exceeds 7 or 8 per cent. These findings are of interest primarily to the investigator concerned with the pharmacology and mode of action of penicillin. For the physician, the uncorrected concentrations may be more useful since such values probably more nearly represent the effective bactericidal activity of a given penicillin.

The experimental data here reported may explain, at least in part, the discrepancy between apparent failure of penicillin to reach the cerebrospinal fluid in man and the profound therapeutic response frequently obtained in disease, as for example, in neurosyphilis. They suggest that effective concentrations of penicillin may be present, but not demonstrable by current *in vitro* methods of bioassay because of the inhibitory effect of cerebrospinal fluid itself on the assay.

SUMMARY

Human cerebrospinal fluid exerts a hitherto unreported inhibitory effect on the bactericidal activities of penicillins G, X and K, *in vitro*, as determined by a serial dilution bioassay method.

This effect is the same for the 3 species of penicillin studied and is comparable in magnitude with that exerted by human serum on penicillins G and X (not penicillin K). Preliminary evidence suggests that protein binding is not the sole, or even an important, mechanism involved, but that there may be actual destruction of penicillin by the cerebrospinal fluid.

Corrective factors are tabulated which compensate for the inhibitory effect of human cerebrospinal fluid on the *in vitro* bioassay of penicillins G, X and K.

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ESTIMATION OF ACID PHOSPHATASE OF HEMOLYZED SERUM BY THE FORMALDEHYDE INACTIVATION TECHNIC*

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Human red blood cells contain large amounts of an acid phosphatase which acts readily upon monophenylphosphate. Estimation by the Gutman technic⁴ has shown the acid phosphatase activity of red cells to be more than one hundred times that of normal serum.⁵ Hemolysis is, therefore, an important source of error in determinations of serum acid phosphatase by this method. Abul-Fadl and King,^{1, 2} using a modified Gutman technic, found that inclusion of 0.5 per cent formaldehyde in the buffer-substrate-serum mixture completely inactivated red cell phosphatase without affecting prostatic phosphatase. Our experiences with the use of this test are here reported.

TECHNIC

Formaldehyde inactivation involved the following changes in the Gutman technic. A formaldehyde reagent was prepared daily by placing 10 ml. of 40 per cent formaldehyde (analytic reagent grade) and 1 drop of 1 per cent alcoholic solution of phenolphthalein in a test tube graduated at 20 ml., adding 0.1 N sodium hydroxide until a faint, permanent pink color was obtained and then diluting with distilled water to a total volume of 20 ml. In setting up the formaldehyde inactivation test, 0.25 ml. of this formaldehyde reagent was added to the 10 ml. of buffer-substrate solution before the addition of 0.5 ml. of serum. After incubation at 37 C. for three hours, 4.25 ml. (instead of 4.5 ml.) of diluted phenol reagent was added. Otherwise, the reagents, procedure and calculations were exactly as described by the Gutmans.⁴ It was found that inclusion of the formaldehyde reagent and reduction of the volume of diluted phenol reagent had no effect on color development of the controls.

Effect of Hemolysis on Acid Phosphatase Activities of Serums

Bloods were collected with the usual precautions to prevent hemolysis. A part of each sample was left undisturbed to clot, and the serum, free from hemolysis, was separated from the clot. The remainder was shaken during clotting, the clot broken up and the hemolyzed serum separated. In all experiments the degree of hemolysis of the latter specimen was greater than that usually met in routine blood samples. Estimations were made of the acid phosphatase of the hemolyzed and nonhemolyzed serums with and without formaldehyde inactivation.

* This work was done with the aid of a grant from Mr. T. Howard Stewart, a Governor of the Montreal General Hospital. Received for publication, May 10, 1948.

The results are shown in Table 1. Comparison of the values obtained without formaldehyde inactivation shows the magnitude of the errors introduced by hemolysis. The close agreement between the values obtained with formaldehyde inactivation demonstrates the inhibition of red cell phosphatase and elimination of errors due to hemolysis.

TABLE 1
ACID PHOSPHATASE ACTIVITIES OF HEMOLYZED AND NONHEMOLYZED SERUMS WITH AND WITHOUT FORMALDEHYDE INACTIVATION

EXPERIMENT NUMBER	ACID PHOSPHATASE (GUTMAN UNITS PER 100 ML. OF SERUM)				DIAGNOSIS
	With Formaldehyde Inactivation		Without Formaldehyde Inactivation		
	Hemolyzed Serum	Nonhemolyzed Serum	Hemolyzed Serum	Nonhemolyzed Serum	
1	1	1	5	2	No disease
2	1	1	5	2	No disease
3	1	1	5	2	Hepatitis
4	2	2	5	3	Cirrhosis of liver
5	3	2	6	3	Prostatic carcinoma
6	2	2	8	4	" "
7	4	4	18	6	" "
8	7	6	18	8	" "
9	9	10	15	10	" "
10	11	11	16	13	" "
Average	4	4	10	5	

TABLE 2
NORMAL VALUES OF SERUM ACID PHOSPHATASE WITH AND WITHOUT FORMALDEHYDE INACTIVATION
(Based on analyses of 70 nonhemolyzed serums)

ACID PHOSPHATASE (GUTMAN UNITS PER 100 ML. OF SERUM)		
With Formaldehyde Inactivation		Without Formaldehyde Inactivation
Maximum.....	3	4
Minimum.....	1	1
Average.....	1.6	2.6

Normal Values of Serum Acid Phosphatase After Formaldehyde Inactivation

Abul-Fadl and King^{1,2} found that formaldehyde not only completely inactivated red cell phosphatase, but also partially inhibited the acid phosphatase of normal nonhemolyzed serums. Evidence of this is seen when values obtained with nonhemolyzed serums with and without formaldehyde inactivation are compared (Table 1). To determine the extent to which normal values are affected by formaldehyde, we estimated the acid phosphatase activities after formaldehyde inactivation of 70 nonhemolysed serums with normal total acid phosphatase

activities.† The results (Table 2) show the normal range after formaldehyde inactivation to be 1 to 3 units per 100 ml. of serum and the average fall in activity due to formaldehyde to be 1 unit per 100 ml. of serum.

Abul-Fadl and King² recommended the routine use of this procedure in all estimations of serum acid phosphatase. However, since the original Gutman technic was found to be satisfactory in our laboratory, formaldehyde inactivation was used only in hemolyzed serums.

SUMMARY

Attention is drawn to the use of inactivation by formaldehyde of phosphatase of red blood cells in order to eliminate errors due to hemolysis in estimation of serum acid phosphatase by the Gutman method. The technic is described, the effect of hemolysis on the acid phosphatase activities of serums is discussed and normal values are presented.

Acknowledgment. Grateful acknowledgment is due to Professor E. J. King, of the British Postgraduate Medical School, who very kindly sent us details of the formaldehyde inactivation technic prior to their publication.²

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† In our experience the normal range without formaldehyde inactivation is 1 to 4 units per 100 ml. of serum.³

A MODIFICATION OF THE BREWER ANAEROBIC JAR*

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The need for a versatile anaerobic culture apparatus in the medical laboratory was demonstrated during the recent war. Military demands called for equipment that could be adapted to varied laboratory situations in overseas areas and in the United States. These demands required an apparatus that would be suitable for simple anaerobic technics as well as the more refined procedures described by Brown,^{3,4} Brewer,¹ Weiss and Spaulding⁵ and Spaulding and Goode.⁵ A survey of the existing apparatus suggested that minor changes in the Brewer anaerobic jar would accomplish this purpose.

The alterations were directed towards fitting the jar for technics requiring an evacuation-replacement system in addition to the technics previously described. In brief they consisted of modifying the outlet nipple to a tapered, gas-jet type for better connection with the source of negative pressure. This tapered nipple permitted the use of rubber tubing of varied diameters, since the exact size required might not be readily available in every military installation. The under surface of the metal lid was ground to effect an airtight seal with the ground glass surface of the jar. The rim was machined so that the lid would slide off like any ground surface vacuum jar. A leak-proof lid called for metal that was impervious to air or could be rendered so by proper treatment. Several lids were cast in lighter alloys and machined to the proper surface. These lids seemed satisfactory, but on prolonged standing the porosity of the castings began to manifest itself. Several methods of impregnation with various materials were tried, but none was found which could be depended on to render the casting safe over extended periods of time. We, therefore, returned to the original bronze which, although quite heavy, was satisfactory from the standpoint of porosity. To withstand the negative pressure encountered during evacuation, a heavy-duty glass jar was substituted. Specifications called for a jar that would tolerate evacuation to 730 mm. negative pressure and resist the thermal shock occasioned by repeated autoclaving or heating immediately adjacent to the lid. Celloseal (Fisher), as used by Weiss and Spaulding, was a satisfactory sealing agent.

To test this apparatus, trial cultures were made in the Bacteriology Department, Army Medical School, Washington, D. C. All standard strains of the clostridia grew as luxuriantly as with any other method tried. All species of clostridia, including *Clostridium tetani*, encountered in infected wounds were cultured, as well as many nonpathogenic organisms. As a further test for main-

* Received for publication, March 12, 1948.

† Present address: George Washington University Hospital, Washington, D. C.

tenance of anaerobic conditions, methylene blue indicator solution was placed in the jar. The indicator was reduced and remained so for the fourteen days during which the experiment was in process.

In many laboratories where hydrogen is not available, manufactured, natural or bottled gas may be readily obtained. The technic, described by Brewer and Brown² for using illuminating gas in the apparatus devised by Brown, Fildes and McIntosh, or in other anaerobic jars which utilize the Laidlaw principle, may be employed. Using such technic, we were able to grow the following anaerobes which were supplied by Dr. R. S. Spray:*

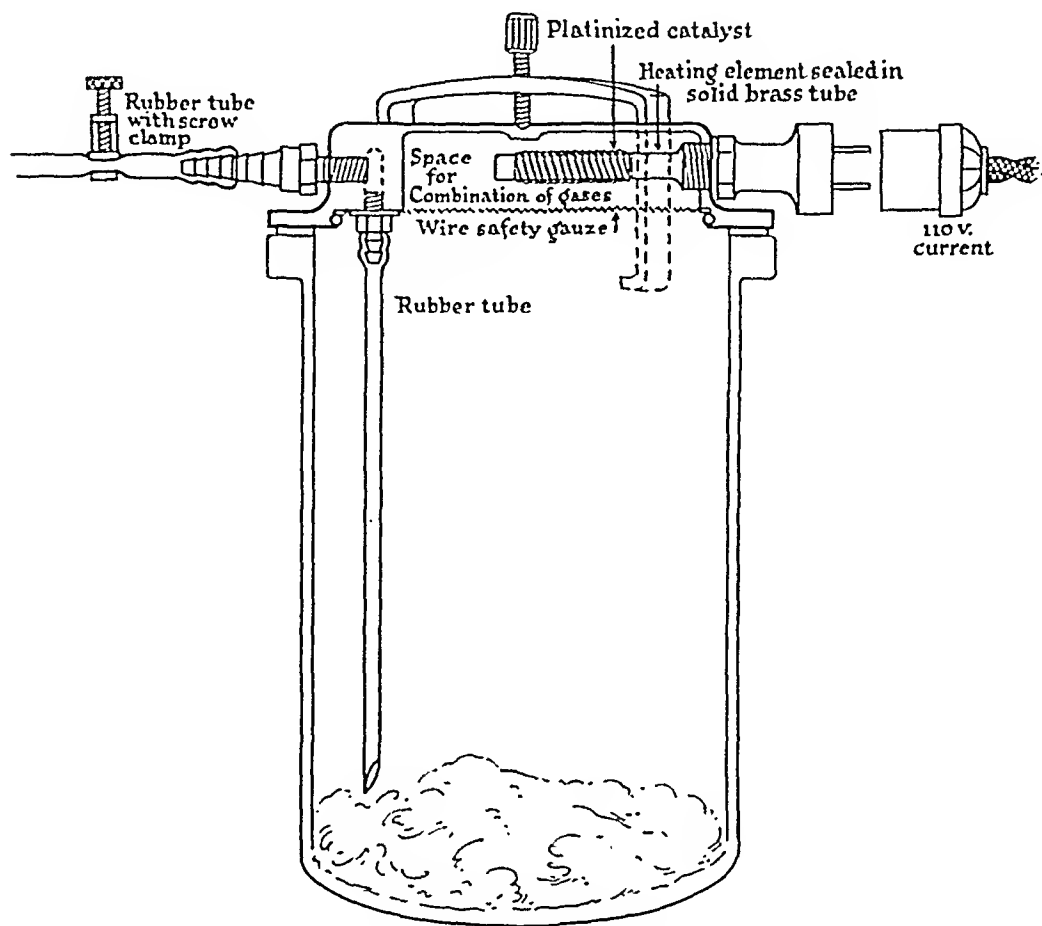


FIG. 1. Illustration of Cross-Section of Jar Showing Construction

Clostridium perfringens, *Cl. tertium*, *Cl. botulinum* A, *Cl. botulinum* B, *Cl. botulinum* C, *Cl. sporogenes*, *Cl. histolyticum*, *Cl. tetani*, *Cl. putrificum*, *Cl. bifermentans*, *Cl. septicum*, *Cl. multifementans*, *Cl. norji*, *Cl. sordelli* (2 strains), *Cl. sphenoides*, *Cl. tyrosinogenes*, *Cl. pasteurianum*, *Cl. butyricum*, *Cl. beijerinckii*, *Cl. acetobutylicum*.

The medium used was infusion broth base with 1 per cent dextrose and 0.075 per cent cystine, at pH 7.6.

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The modified apparatus* sacrifices none of the features of the Brewer anaerobic jar, the advantages of which have been fully discussed.¹ In addition, the scope of the culture jar has been extended to permit its ready use with carbon dioxide in simple evacuation-replacement procedures and in evacuation-replacement technics employing catalysts. In one of the latter methods, described by Weiss and Spaulding,⁶ the hazard of explosion of the oxygen-hydrogen mixture is minimized since the amount of oxygen in the jar after evacuation is negligible. Also, preliminary evacuation expedites the preparation of cultures since the time required with this technic is but five to ten minutes.

Little ingenuity would be required to adapt the jar for use in situations where electrical current, vacuum pumps and other laboratory conveniences are not available. A fair degree of evacuation can be achieved by a suction pump attached to a water faucet. In the absence of electric current the finely divided palladinized asbestos is a sufficiently active catalyst to render heating unnecessary. Accordingly, after preliminary evacuation, the remaining oxygen can be removed by the palladinized asbestos in the lid. To reactivate the catalyst after repeated use, the entire lid may be heated in an oven and allowed to cool.

SUMMARY

A modification of the Brewer anaerobic jar is described. The new features embodied are a tapered, gas-jet type, outlet nipple, and a cast bronze lid which is impervious to air and is ground on its under surface to effect an airtight seal with the ground glass surface of the jar. The glass jar specified is resistant to thermal shock and will tolerate evacuation to 730 mm. negative pressure. The revised jar can be used for any of the anaerobic culture methods based on the Laidlaw principle, with evacuation-replacement procedures, or in methods representing a combination of these technics. It can be utilized with manufactured, natural or bottled gas, or with carbon dioxide.

Acknowledgment. It is a pleasure to acknowledge the cooperation and technical assistance of Mr. Theodore J. Carski, Baltimore Biological Laboratory, Baltimore, Maryland.

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* The jar is available from the Baltimore Biological Laboratory, Baltimore, Maryland.

A METHOD FOR THE STUDY OF AERIAL AND REPRODUCTIVE MYCELIA*

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A review of the literature shows several methods for the study of molds. These include such methods as the microslide,^{1, 2} the single-spore culture tube³ and the platform culture.⁴

The present method uses glass rods which are drawn out over a Bunsen flame to a diameter of 0.5-0.75 mm. Upon cooling they are cut in lengths of approximately 22 mm. The rods are then stuck in radial fashion into a small pyramid of modeling clay (Permoplast)[†] whose base is pressed onto the floor of a petri dish (Fig. 1a). The glass rods are best manipulated when they are inserted

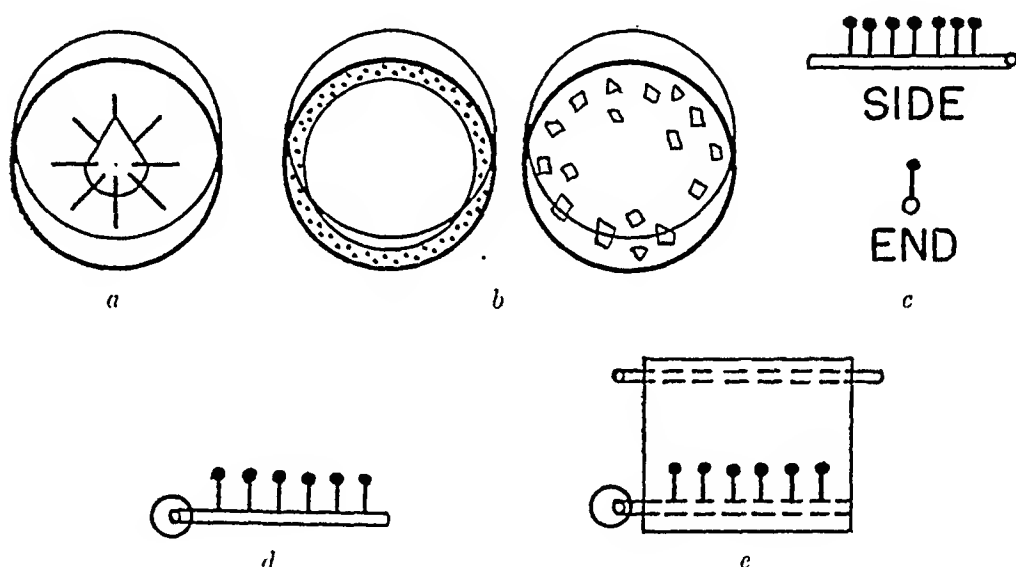


FIG. 1.

midway between the base and apex of the clay pyramid. Around the edge of the petri dish floor, a rim of blotting paper 10 to 15 mm. wide or filter paper cut in small pieces and moistened with water, serves to maintain sufficient humidity for the best growth of the mold (Fig. 1b). Sterilization of the petri dish, the paper and the clay pyramid is effected by autoclaving from ten to fifteen minutes at 121 C. under 15 pounds pressure.

A small tube of Sabouraud's agar is then melted, cooled to 50 C. and heavily inoculated with spores of the mold to be examined. While the agar is still fluid, a wire loop is dipped into it and then so drawn along the rod surface as to be tangent to it and in the same plane at all times. This prevents overloading the rod with agar and is important in that the number of spores present is de-

* Received for publication, June 1, 1948.

† Obtainable from American Art Clay Co., 4717 W. 16th Street, Indianapolis, Indiana.

pendent on the amount of agar used on each rod. The less agar on a rod, the fewer the spores present and, consequently, the less crowded will be the growth of mold after incubation. Thus, it is possible in a sparse field to examine the morphology of each aerial mycelium individually.

After the incubation period, the aerial mycelia of the mold are seen growing perpendicularly to the circumference of the glass rod and along the tangent plane upon which the agar was originally spread (Fig. 1c). At this time, the culture rod is removed from the pyramid, rotated 90 degrees on its longitudinal axis and fastened to a microscope slide by means of a small bit of the clay placed between rod and slide, at one end (Fig. 1d). Examination at this point shows the aerial mycelia extending perpendicularly from the rod and parallel to the slide. A clean rod is now placed on the slide parallel to the first and approximately 15 mm. from it. After a drop of Amann's solution* is added, the coverslip is supported by the two rods. The mold is now ready for examination (Fig. 1e).

Up to the present, molds examined by this method have been species of *Penicillium*, *Aspergillus* and *Rhizopus*. The aerial and reproductive hyphae are clearly defined against the blue background.

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* 20 Gm. phenol, 20 ml. lactic acid syrup, 40 ml. glycerol, 20 ml. water, .05 Gm. Cotton Blue.

A MODIFIED CONWAY HORIZONTAL MICRO-BURET*

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In the adaptation of micro-diffusion technic to analytic problems on the ultra-micro scale, Conway¹ perfected a horizontal micro-buret, which has an accuracy equal to that of the Relberg apparatus but possesses considerably greater ease

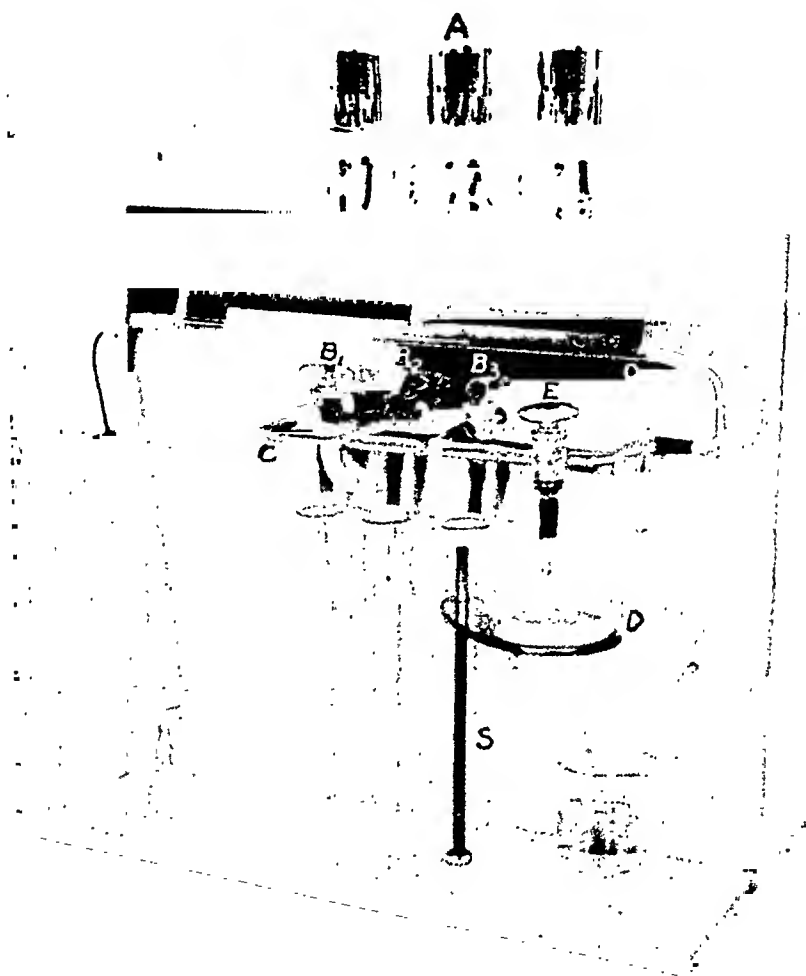


FIG. 1.

of manipulation. This buret facilitates the speed and convenience of the micro-diffusion technic. Since there is no such instrument available in this country, it was necessary to construct one. During the construction certain modifications were incorporated which increased the adaptability of the instrument.

One inconvenience of the original apparatus was that it employed a single reservoir for the standard titrating fluid. This necessitated either using a series of

* Received for publication, June 10, 1943.

burets to cover the various ranges of titration or altering the standard to obtain a change of titrating range. To obviate this disadvantage of manipulation there was introduced a system of three reservoirs (Fig. 1) containing different standards so that titration ranges could be readily interchanged. The three reservoirs (A), which are situated on an attached platform above and behind the buret, are connected by glass tubing through three-way stopcocks (B_1, B_2, B_3) to a manifold (C) which connects with the horizontal buret (O) and the titrating stopcock (E). The system is filled from any of the three reservoirs by gravity. To change from one standard to another, *e.g.*, from N/1000 HCl to N/100 HCl, it is imperative to flush out the system thoroughly with the replacing fluid. This is accomplished by adjusting the stopcocks of the two inactive reservoirs to the drainage position and then filling the system from the "active" reservoir. After closure of the stopcocks of the "inactive" reservoirs, the buret is filled and emptied through the titrating cock three times. The buret is then ready for use.

Since the rate of delivery from this buret is controlled largely by the size of the end-point emergence, gravity being constant in its effect, the delicacy of titration depends ultimately on this factor. The end-point emergence is constant for each tip and for accurate work should be minimal. It is, therefore, the structure and orificial diameter of the titrating tip which determines the fineness of control of the meniscus, *i.e.*, whether the end-point emergence requires a fluid volume of less than, or more than, a scale division. It may be necessary to draw many tips before one of suitable quality is obtained. Because of this it is desirable to employ exchangeable tips, which is most conveniently done by snugly fitting the tips to the stopcock with gum-rubber tubes. No difficulty with air traps is encountered if the tip is filled prior to being fitted. The principle of the exchangeable tip is of great value in the standardization of the buret where a heavy liquid is used and emergence control by standard aperture is very difficult. Provision for variation in length of tip and immersion of the tip may be made by an adjustable platform (D) which is easily elevated and lowered. The upright (S) of the platform may also serve to support clamps for test tubes and flasks.

The buret may be made from such tubing as precision-bored, 1 mm. tubing, thermometer tubing, and even standard soft glass capillary tubing, of about 30 cm. length, to accommodate a volume of 0.20 ml. This is strapped into position (O) with a background of an accurate 25 cm. steel scale (F) graduated down to 0.05 cm., which corresponds to about 0.5 cu. mm. in volume. The positions of the scale ends are etched in the tubing, and the tubing is calibrated against the scale with mercury. The readings are plotted as a graph. This obviates the need of marked tubing and permits versatility in the selection of length and bore of tubing, although the marked tubing has certain advantages.

Acknowledgment. The authors wish to express their thanks to Professor E. J. Conway, F.R.S., for his interest and comments on the construction of this model of the buret.

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USE OF SILICONE-TREATED NEEDLES IN BLOOD DONATION*

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The occurrence of clotting during the collection of blood for transfusion is a source of considerable trouble. In the majority of instances the clotting process commences within the lumen of the intravenous needle. Large and small clots may pass along the rubber tubing into the bottle. Use of larger gauge needles has partially obviated this trouble, but, owing to the technical difficulties of insertion into small veins, this practice is not entirely satisfactory.

In 1946 Jaques and his colleagues² reported the use of a silicone (General Electric Dri-Film No. 9987) in the experimental prevention of clotting of blood. Coagulation was inhibited without the use of anticoagulants for long periods (one and one-half to six hours) in whole blood in contact with silicone-treated surfaces.

In an attempt to reduce the clotting in the collection of blood for transfusion, intravenous needles were treated with silicone and the results compared with untreated needles under controlled conditions. Comparisons were made in two separate clinics held on two different days. One nurse-technician performed all the venipunctures for each clinic, using a standardized technic. The criteria for comparison were:

1. Assessment of the average rate of flow per 100 ml. of blood, calculated by stop watch and measured from the time of insertion of the needle to the completion of the donation.
2. The number of incompletely filled bottles.
3. The number of bottles containing visible clots after the erythrocytes had settled.

Equipment used was the standard blood collection sets of the Canadian Red Cross Blood Transfusion Service, which are essentially the same as the equipment of the Medical Research Council of Great Britain as described by Brewer.¹ Blood was collected by gravity flow, no vacuum being used. The intravenous needles were gauge 15 with hollow-ground medium bevels.

G. E. Dri-Film is supplied in liquid form. On exposure to the surface, hydrochloric acid is released and must be washed free. The needles were treated in the following manner: Dri-Film (silicone) was "synergized" through the needles. The excess silicone was removed with a cloth-covered, motor-rotated stylet, and the needle was then synerized with pyrogen-free, sterile, distilled water and repolished. After assembly, the taking sets were autoclaved (25 pounds for thirty minutes). Autoclaving had no visible effect on the efficiency of the silicone coating in the maintenance of a dry surface.

* Received for publication, June 1, 1948.

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The results of the experiment are shown in the following table:

	ELAPSED TIME IN SECONDS	VOLUME OF BLOOD COLLECTED (ML.)	RATE PER 100 ML. IN SECONDS	NO. OF DONORS			BOTTLES IN COMPLETELY FILLED	BOTTLES WITH VISIBLE CLOTS
				Male	Female	Total		
A. Untreated Needles								
Clinic #1.....	15,858	17,270	91.8	32	16	48	9	17
Clinic #2.....	15,726	17,350	90.6	29	17	46	2	12
Totals.....	31,584	34,620	91.2	61	33	94	11	29
B. Treated Needles								
Clinic #1.....	13,046	16,400	79.5	34	12	46	2	5
Clinic #2.....	13,360	16,650	80.2	34	11	45	2	5
Totals.....	26,406	33,050	79.9	68	23	91	4	10

RESULTS

Blood was collected in 94 bottles, using untreated needles. Eleven bottles were incompletely filled and 29 contained visible clots. Blood was also collected in 91 bottles, using silicone-treated needles. Four bottles were incompletely filled and 10 contained visible clots. There was, therefore, a significant and consistent improvement in yield with the use of silicone-treated needles.

There was also significant increase in the rate of flow. Remarkably consistent net rates per 100 ml. of blood are shown for each clinic with both treated and untreated needles. The rate of flow through the silicone-treated needles was 11.3 seconds per 100 ml. faster than through the untreated needles.

With untreated needles, clotting in the rubber tubing on completion of the donation was frequently so rapid that specimens for Kahn and serologic tests were obtained with difficulty. No clotting occurred with the silicone-treated needles, and full specimens were collected easily and rapidly.

SUMMARY

Silicone treatment of surfaces for the prevention of clotting has been applied to intravenous needles in the collection of blood donations. Using three criteria: rate of flow, occurrence of visible clots and the number of incompletely filled donations, there was consistent improvement with the treated needles in a controlled comparison with untreated needles.

Silicone treatment of glass and other surfaces has a number of practical applications in both technical laboratory procedures (where paraffin or vaseline was formerly used) and in clinical technics, particularly in the prevention of clotting within cannulas and syringes, as in exchange transfusions in the treatment of erythroblastosis fetalis.

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A RAPID METHOD FOR PARAFFIN SECTION STUDY OF EXFOLIATED NEOPLASTIC CELLS IN BODILY FLUIDS*

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Hunter and Richardson¹ have recently outlined an excellent concentration method of bodily fluids for preparation of paraffin sections. They precipitate the proteins by picric acid, filter the specimen and then utilize a Papanicolaou staining technic. They believe that paraffin sections are more satisfactory than smears for the demonstration of neoplastic cells in fluids. We also have found, on several occasions, that paraffin sections were definitely more instructive than smears prepared from the same specimen. However, if a paraffin method is to be employed, it must be simple and rapid in order to be useful in routine work. With the aid of the Autotechnicon it is readily possible to prepare suitable sections of various fluids within one day.

PROCEDURE

The fluid is concentrated by centrifugation, and the sediment is mixed with powdered fibrinogen† in a small evaporating dish. This material, which dissolves readily, is added rather freely. A small amount of powdered thrombin‡ is added, and the resultant coagulum is fixed, embedded, sectioned and stained in the usual way. One may use whatever stain he desires, but we have found that hematoxylin and eosin is satisfactory.

By use of this method we have demonstrated the presence of desquamated cancer cells in pleural and ascitic fluids, bronchial aspirations, sputum and urine. The technic is not applicable to all fluids, but where applicable is extremely satisfactory. We utilize it only for thinner fluids that may be concentrated by centrifugation. We have not attempted to employ it in the study of gastric or vaginal secretions, and it is not very satisfactory for tenacious sputum.

In practice, upon receipt of a fluid specimen in the laboratory, the pathologist or the resident in pathology inspects it and utilizes the method or methods which seem most adaptable to the particular specimen. Smear, picric acid concentration and centrifugation technics are all utilized as indicated by the nature of the fluid, for one's objective is to obtain a preparation in which neoplastic cells, if present, are well demonstrated.

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† Preparations used are Thrombin (bovine), The Upjohn Company, Kalamazoo, Michigan, and Fibrinogen (bovine), Armour and Company, Chicago, Illinois.

ELECTRICAL BLOOD COUNTER*

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Few laboratory tests are more monotonous than blood counting. Monotony endangers accuracy. But sometimes a mechanical, repetitive operation which the mind rejects as monotonous is readily accepted by the fingers as, for example, knitting or telegraphy.

The commonly available finger-operated blood cell counters have been tried by the Clinic's technicians and discarded because the operation was slow or fatiguing or because of other mechanical reasons. Instead, an apparatus was assembled in which electricity furnished the operating force, thus permitting speed of operation; and the fingers applied only



FIG. 1. ELECTRIC BLOOD COUNTER

trigger force through a telegraph key, which is one of the least fatiguing of manual instruments.

The apparatus (Fig. 1) consists of an electrical impulse counter of the kind ordinarily used in industry to record the operations of automatic machines,† a "microswitch" and a telegraph key mounted on a rubber-bottomed metal base. The key carries no current, but acts only as a mechanical lever to operate the microswitch, inasmuch as live keys carry current on uninsulated metal, which was considered unsafe in a laboratory.

The apparatus, which is illustrated, has been in use for a year without requiring further alterations. It has added interest to a dull method, has reduced the counting time to less than one-half, and has unexpectedly reduced the interruptive effect of external stimuli. The only disadvantage so far recognized is that of the initial cost.

* Received for publication, May 21, 1948.

† This part of the apparatus is manufactured by Veeder-Root, Inc., Hartford, Connecticut. One should specify model US, "manual turn-back knob", and indicate the direction, voltage and cycles of available current.

A SUGGESTED LABORATORY TURNTABLE*

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The turntable, here illustrated, is offered as a simple mechanical device which will permit rotation of a petri dish while keeping it anchored.

This device will allow one hand to rotate the dish and to hold the cover in position to prevent contamination, thus leaving the other hand free for spreading cul-

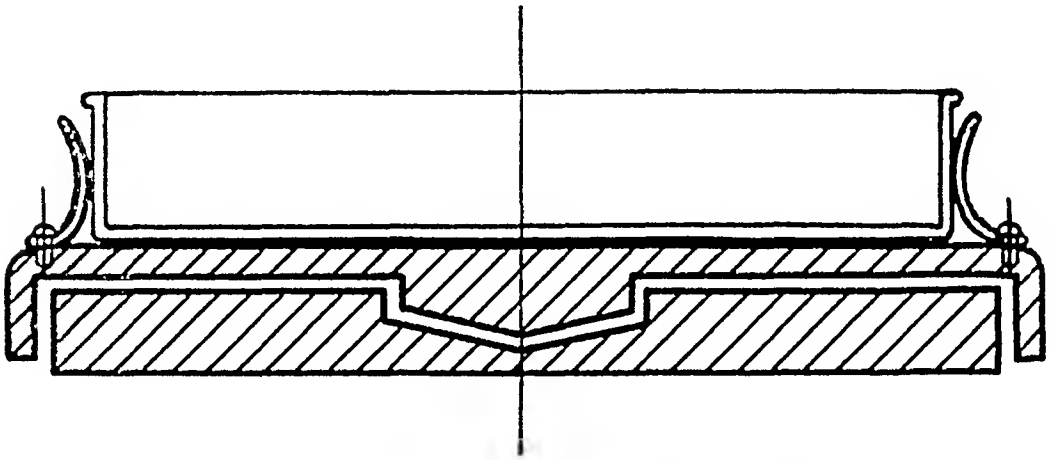


FIG. 1. Cross section of laboratory turntable showing bottom of petri dish in position.

tures, picking colonies and changing position of specimens. Since its secure position prevents the dish from being moved along the laboratory work bench there is less likelihood of accidental spilling or contamination. Desirable features of such an apparatus are a maximum height of one-half inch, material that will withstand any necessary sterilization and that is heavy enough to prevent accidental dislodging, and adjustable clips to provide for variation in size of dishes.

* Received for publication, May 20, 1918.

HEMOLYTIC ANEMIA WITH HEMOGLOBINURIA*

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Hemolytic anemia with hemoglobinuria is frequently a striking clinical event indicative of extensive intravascular hemolysis. The condition lends itself to detailed study because of the morphologic abnormalities in the blood and bone marrow, the marked accumulation of hemoglobin and its derivatives in the blood plasma and urine, the aberrations of renal function incident to the hemolytic process and the variety of pathogenetic mechanisms which may give rise to it. During the past ten years we have had an opportunity to study carefully a large number of cases of hemoglobinuria; these are reported in this communication. Our cases do not include all the described varieties of hemoglobinuria, but direct attention to the types that occur in general civilian medical practice in temperate climates. They fall into four groups, namely, those presenting the Marchiafava-Micheli syndrome (paroxysmal nocturnal hemoglobinuria), those caused by drugs or cold hemagglutinins and an idiopathic variety. No attempt will be made in this paper to deal with the hemoglobinurias that we did not observe personally, such as hemoglobinuria of blackwater fever, favism, burns, bartonella infections, sepsis, snake bites, spider bites, syphilitic paroxysmal cold hemoglobinuria, myoglobinuria, march hemoglobinuria, hemoglobinuria following the intravenous infusion of distilled water or transfusions of incompatible blood or plasma and hemoglobinuria associated with the action of certain chemical agents.

At the outset it is necessary to distinguish clinically between the hemoglobinurias and the larger group of hemolytic anemia without hemoglobinuria. In both, excessive destruction of red blood cells occurs, morphologic findings in the blood and bone marrow are similar and increased excretion of urobilinogen is the rule. The rationale for differentiation rests upon the probability of fundamental difference in the mechanisms of the hemolysis and, as discussed subsequently, the inapplicability of splenectomy in the treatment of hemoglobinuria. In some of the hemolytic anemias without hemoglobinuria the spleen occupies a cardinal position in the hemolysis. This is proved by the cessation of excessive hemolysis following splenectomy. In the hemoglobinurias, on the other hand, hemolysis is either initiated differently or proceeds in another location (*e.g.*, the circulating blood), and the dominance of the spleen is not apparent. The hemoglobinuric states in which this concept is proved are:

1. Paroxysmal nocturnal hemoglobinuria in which splenectomy does not stop the hemolytic process.
2. Paroxysmal cold hemoglobinuria due either to cold hemagglutinins or to syphilis; hemoglobinemia can be shown to be a local process occurring in the blood of the chilled part.
3. Incompatible blood transfusions.

* Presented in abstract at the Twenty-Sixth Annual Meeting of the American Society for Clinical Pathologists, in Chicago, October 29, 1947. Received for publication, May 15, 1948.

Certain acute and chronic hemolytic anemias, for example, chronic familial hemolytic anemia and sickle cell anemia, no matter how severe, are never, in our experience, attended by hemoglobinuria or hemoglobinemia. On the other hand, the hemolytic anemias in the hemoglobinuric group, even when quite mild, were regularly associated with hemoglobinemia. Since investigation of the blood plasma for hemoglobin in hemolytic processes is usually not made unless there is hemoglobin in the urine, it is likely that if the hemoglobinemia had been looked for, some hemolytic anemias without hemoglobinuria would more properly be classified with the hemoglobinurias. Hemoglobinemia has the same significance as hemoglobinuria; the absence of the latter finding merely indicates that the plasma level of pigments was not high enough to pass the renal threshold. In some cases caused by drugs, hemoglobinuria could not be found, but hemoglobin was present in the plasma. Similar findings were not uncommon in the hemolytic anemia associated with incompatible blood transfusion, especially when the reaction was mild. In another type of hemolytic anemia in patients with potent cold hemagglutinins, neither hemoglobinemia nor hemoglobinuria may be observed. As we indicate later, in some cases of this kind it may be possible to produce hemoglobinemia by immersion of an extremity in ice water. Our observations in hemolytic anemia without hemoglobinuria were not always complete enough to establish the cases which should be classified with the hemoglobinuric cases on the basis of the presence of hemoglobinemia or of its production by a clinical test.

In Table 1, a comparison is given of certain similarities and differences in the hemoglobinurias we have examined. Certain comments are necessary to explain these findings. It will be noted that except for those unusual instances in which potent cold hemagglutinins were present, a chronic anemia with episodes of hemoglobinuria over periods of weeks or years occurred only in the Marchiafava-Micheli type. This important consideration loses some of its significance, however, when it is realized that hemoglobinuria may appear infrequently in this disease and may be unnoticed by the patient. We have observed cases in which hemoglobinuria was noted less than one day a year. On the other hand, if greater dependence were placed upon examination of the blood plasma for hemoglobin pigments this difficulty was overcome, for it was only in the Marchiafava-Micheli type that persistent hemoglobinemia occurred. We have never failed to find oxyhemoglobin or methemalbumin in the plasma of patients with this disease. In the other hemoglobinurias the same pigments were found in the plasma, but only for a short period of time, rarely exceeding one week, during which time the acute phase of the disease had subsided and recovery had started.

Hemoglobinemia and Hemoglobinuria

The simplest and at the same time the most accurate method for the detection of hemoglobinemia or hemoglobinuria is by spectroscopic examination of the plasma or centrifuged urine. No clinical laboratory can be considered to be completely equipped if it does not have facilities for this test. The test can be performed satisfactorily with a hand instrument, especially if a wavelength

scale in the visible range accompanies the spectrum. With the proper adjustment of the intensity of the light, the thickness of the specimen and the

TABLE 1
DIFFERENTIATION OF MAIN TYPES OF HEMOGLOBINURIA

CLINICAL ASPECTS	MARCHIAFAVA-MICHELI SYNDROME	DRUGS	COLD HEMAGGLUTINATION	IDIOPATHIC
Duration of disease	Many years	Transient	Transient or chronic	Transient
Splenomegaly	Slight to marked	Slight or absent	Slight or absent	Slight or absent
Icterus	Persistent	Transient	Transient	Transient
Hemoglobinuria	Intermittent; absent to marked	Transient; marked	Transient or intermittent; absent to marked	Transient; marked
Hemoglobinemia	Permanent	Transient	Transient	Transient
Hemosiderinuria	Always present	Rare	Rare	Rare
Renal function	Not impaired	May be severely impaired	Not impaired	Not impaired(?)
Complications	Thromboses; biliary calculi; purpura	Uremia	Raynaud's syndrome; gangrene of extremities	None
Response to transfusions	Fair; febrile and hemoglobinuric reactions	Good	Good	Good
Recurrences	Invariable	No, unless drug is given again	Yes, in occasional cases	Yes, in occasional cases
Blood picture				
Anemia	Mild to severe; chronic	Severe acute	Absent to severe	Mild to severe; acute
Red cells	Macrocytosis	Spherocytosis	Spherocytosis mild or absent	Spherocytosis
White cells	Leukopenia	Leukocytosis	Leukocytosis	Leukocytosis
Platelets	Thrombocytopenia	Thrombocytosis	Thrombocytosis	Thrombocytosis
Reticulocytosis	Slight to moderate	Slight to marked	Absent to marked	Absent to marked
Diagnosis	Positive acid hemolysis; positive heat resistance; perpetual hemosiderinuria	History; fragmentation of red cells; Heinz bodies	High titer cold hemagglutinins; cold hemolysis with shaking; Ehrlich-Rosenbach test	By exclusion
Prognosis	Favorable for life; recovery never occurs	Guarded, especially after sulfonamides	Favorable	Favorable

addition of several reagents, the common hemoglobin pigments can be recognized readily and a semi-quantitative estimate of their concentration established.

For more accurate work a spectrophotometer or a Hartridge reversion spectro-scope is necessary.

The alpha absorption maximums are most useful for spectroscopy. For oxy-hemoglobin the band is at 576 microns (μ); for methemoglobin, 630 μ ; for methemalbumin, 624 μ ; and for sulfhemoglobin, 618 μ . With the ordinary clinical instruments, the latter three pigments cannot be differentiated without resorting to further analysis. Extracorpuseular sulfhemoglobin rarely if ever occurred in the hemoglobinemias we are discussing. Oxyhemoglobinemia was invariably found in the early stages of intravascular hemolysis. If a band was found near 625 μ , its immediate disappearance and an obvious change in color of the specimen from brown or black to dark red after the addition of a few drops of 5 per cent sodium cyanide, indicated that the pigment was methemoglobin. Methemoglobinuria was common; methemoglobinemia rarely occurred in these hemoglobinurias. If the band was not affected by cyanide but changed its position to 558 μ (ammonium hemochromogen) after the addition of concentrated ammonium sulphide (0.1 ml. to 1 ml. plasma), the pigment was methemalbumin. This was the most commonly occurring pigment in the plasma in these conditions and was often present after the subsidence of active hemolysis when oxyhemoglobinemia had disappeared. The ammonium sulphide test is historically an old reaction known as Schumm's test, which, apparently is now seldom employed in hematology. In our hands it has proved valuable for the detection and differentiation of pigments. Under certain circumstances, when an absorption band was not visible in the neighborhood of 625 μ , the addition of ammonium sulphide caused the appearance of the 558 μ band and, even when the band was visible in the former position, it was usually denser and more easily seen in the latter. We will not go into the question of Schumm's test on plasmas from other diseases, *e.g.*, pernicious anemia in relapse or severe liver disease in which hemoglobinuria does not occur. In all the bloods we examined, only oxyhemoglobin was found in the red blood corpuscles, but not all cases were examined in this fashion. In sulfonamide cases other pigments such as methemoglobin and sulfhemoglobin may be present.

Hemosiderinuria

There are few tests in clinical medicine that are as easy to perform and as reliable as the examination of the urine for hemosiderin. Recent publications in hematology generally do not mention this finding, and it is only lately that we have made it a routine to look for this substance in hemolytic anemias, using the Prussian blue reaction for inorganic iron. There is need for more frequent examinations for hemosiderinuria in hemolytic states. The consistent occurrence of this material in large amounts in the urine is observed only in the Marchiafava-Micheli syndrome, even in the absence of hemoglobinuria. In a number of cases of this disease we have observed its transient absence; in other types of hemoglobinuria or hemolytic anemia we have noted its usual absence or presence only in traces. The factors controlling the excretion of hemosiderin in the Marchiafava-Micheli syndrome are not known except that there may be a tendency toward nocturnal periodicity. This cyclic change is by no means as regular as

the nocturnal increase in the hemoglobinemia. We have also observed this substance in the urine of a patient with aplastic anemia who had received many blood transfusions, and in the urine of a patient with hemochromatosis.

Renal Function

We have not had the facilities to perform complete kidney function studies in these cases of hemoglobinuria. The only instances of seriously impaired kidney function resulting from the hemolytic process were the sulfonamide cases. There is no question that absolute renal insufficiency with anuria or oliguria is common in some hemoglobinuric states like incompatible blood transfusions, sulfonamide and other drug intoxications, blackwater fever and in the crush syndrome, while it is rare in most or all of the other types. The reason for this difference has never been elucidated and the solution of this problem might shed important and fundamental light upon renal pathophysiology. Serious impairment of kidney function has not been observed in any of the chronic or recurrent hemoglobinurias even though the disease may have lasted for many years. Certainly, impairment of kidney function cannot be related directly to hemoglobinuria, and other experience indicates that even the suddenness of the onset of hemoglobinuria and rapid progression of anemia are not necessarily associated with marked nephrotoxic effects.

Serologic Tests

The study of patients with hemoglobinuria by serologic technics is fruitful because the mechanism of the disorder is often uncovered. We, therefore, routinely perform Wassermann or other tests for the presence of syphilis since a positive reaction may lead to the diagnosis of syphilitic cold paroxysmal hemoglobinuria, in which case the Donath-Landsteiner test will also be positive. In the past decade this disease has become extremely rare, and we have not observed a single case. If the history were obtained that hemoglobinuria was related to exposure to environmental cold, the most probable cause was cold hemagglutination. We have previously pointed out the fundamental differences between these conditions. Serologic investigation of the cause of hemoglobinuria must also include the acid hemolysis test of Ham⁴ and the heat resistance test (Fig. 1)⁵ (spontaneous hemolysis of clotted blood at 37 C. in four hours). Positive results in these is unequivocal evidence of the Marchiafava-Micheli syndrome, while negative results exclude this diagnosis. Additional examinations, which are also indicated in hemolytic anemia without hemoglobinemia, must include determination of the Rh type including the subgroups and tests for hemagglutinins against homologous and heterologous erythrocytes of the same blood group in physiologic saline, serum or plasma and bovine albumin mediums. The use of bovine albumin to demonstrate agglutinins as well as the anti-human serum-rabbit serum test (Coombs' test) to demonstrate sensitized erythrocytes have become important only recently and their potentialities have not been explored as yet.¹ Our preliminary observations have indicated negative reactions with the albumin in the Marchiafava-Micheli syndrome (Coombs' test negative in two cases), in one case caused by phenylhydrazine, in one with cold hemagglutinins and in one case of undetermined origin. Finally, hemol-

ysins demonstrable after the addition of guinea pig complement may occasionally be present, although we have never observed their presence.

Blood Picture

Morphologic study of the circulating blood in these patients generally reveals the commonplace evidence of hemolysis as shown by anemia, leukocytosis, normoblastosis of varying degree and reticulocytosis. In one chronic case of paroxysmal cold hemoglobinuria caused by cold hemagglutinins previously reported, none of these criteria was present. Both leukopenia and thrombocytopenia were regular accompaniments of Marchiafava-Micheli disease. In rare cases reticulocytosis was slight or absent throughout the entire course. Extreme thrombocytosis was a feature in one case of polycythemia vera over-treated with phenylhydrazine. Normal platelet counts were frequently present.

Our notes on the occurrence of spherocytosis and macrocytosis of the erythrocytes reveal information of interest that cannot be systematized completely. Just as in certain other types of hemolytic anemia without hemoglobinuria, the presence or absence of these structural abnormalities was not always predictable or explainable. Particularly as regards spherocytosis, our experience was not consistent with the frequently repeated dictum that, if the blood were examined shortly after the onset of hemolysis, spherocytosis would be found. We have observed normal-shaped red blood cells in very severe acute anemias early in their course and, on several occasions, in patients with potent cold hemagglutinins, we have absolutely excluded such an explanation for the absence of spherocytes. This was accomplished by immersing a constricted finger or an arm in ice water, thereby causing local hemoglobinemia in the chilled part. Examination of the blood directly from the member in question revealed hemagglutination of normocytes. A tendency toward macrocytosis was often found in Marchiafava-Micheli syndrome. This diagnosis must be kept constantly in mind in macrocytic hemolytic anemias. Cases with potent cold hemagglutinins were often free of spherocytes or, if this abnormality were present, it was of slight degree. Most of the other cases of hemoglobinuria that we studied showed spherocytosis, often of extreme degree.

We have observed fragmentation of the erythrocytes (Fig. 2) in several drug and idiopathic cases, but have also noted this in hemolytic anemia without hemoglobinemia. Erythrophagocytosis was not a common finding. Heinz bodies were present in one case caused by intoxication with phenylhydrazine.

Bone Marrow

The bone marrow changes in the hemoglobinurias were similar to those observed in other hemolytic states. Pro-erythroblasts, erythroblasts and normoblasts were markedly increased in addition to generalized hyperplasia of the marrow, as a response to the blood destruction. The degree of this change paralleled the severity of the anemia. In several of the cases with minimal reticulocytosis despite anemia, the normo-erythroblastosis was present just as in cases with high reticulocyte counts. The megakaryocytes were increased

even when thrombocytopenia was present, as in the Marchiafava-Micheli syndrome.

Treatment

We have not attempted splenectomy in the hemoglobinurias. (In three of our patients with paroxysmal nocturnal hemoglobinuria, splenectomy was performed elsewhere without benefit.) We believe that hemoglobinemia and hemoglobinuria are contraindications to splenectomy. Almost invariably, in our experience, in acute cases in which there was proper treatment with blood transfusions, the patients recovered relatively promptly and showed marked improvement in several days or faced the sequelae of renal damage. It was

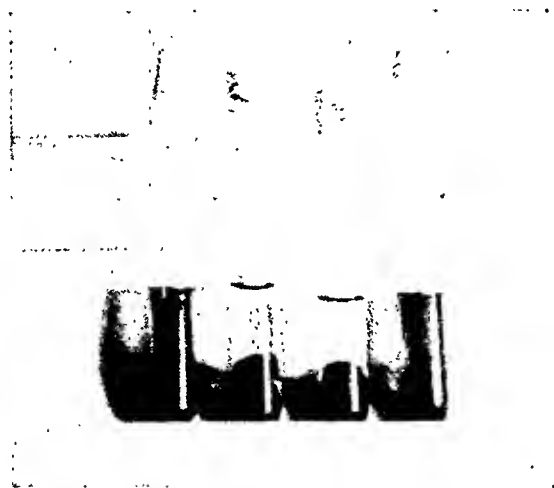


FIG. 1. Color changes in heat-resistance test in Marchiafava-Micheli disease. The two outside tubes show positive reaction after three hours at 37 C. The two center tubes contained the same blood; one tube was kept at room temperature and the other in the ice box for three hours.

distinctly unusual for the hemolysis to proceed at such a rapid pace for so long a period that it became impossible to maintain the blood figures at a reasonable level with transfusions. Even under such an exceptional circumstance splenectomy, in our opinion, should be withheld. It is during the phase of rapid fall in hemoglobin, at and shortly after the onset, that the crucial therapy of frequent blood transfusions is required. Patients with potent cold hemagglutinins were transfused successfully by the usual technic with stored bank blood without any special precautions relative to warming the blood prior to administration. Transfusion reactions did not appear to be any more common in patients with active hemolysis than would be expected in the absence of hemolysis. The only exception to this was in Marchiafava-Micheli disease in which mild febrile reactions were common and exacerbations of hemoglobinuria were occasionally produced. Some patients stated that this appeared to be the case only after

repeated transfusions, but this was variable. These patients usually showed a rise in the blood figures after transfusion despite reactions. In view of this we recommend blood transfusions in this condition only frequently enough to prevent disabling symptoms of anemia. Most of the patients maintain a fairly active life without treatment of any kind.

Our experiences indicate that vigorous transfusion therapy early in the course of acute cases of hemoglobinuria from any cause was the most important measure to insure recovery. In addition, offending drugs must be stopped. After the acute episode the products of hemolysis were removed and, if blood transfusions were not given, alarmingly low levels of hemoglobin resulted. One patient whom we saw after a transfusion of 1000 ml. of blood and after active hemolysis had probably stopped, had a hemoglobin level of 5 Gm. per 100 ml. of blood. When frequent blood transfusions were administered early, the clinical response was sometimes most dramatic. In neglected cases in which severe anemia has not been corrected promptly by transfusion, consideration should be given to the transfusion of blood from which part of the plasma has been removed. In one such patient whom we saw, heart failure was precipitated by the rapid infusion of 1100 ml. of blood. As has been pointed out previously by others and by us, unusual care was required in the blood grouping and cross-matching in patients with potent cold hemagglutinins. Sometimes, the first lead to the diagnosis of potent cold hemagglutination in a patient with hemolytic anemia was provided by the laboratory report of blood group AB or of difficulty in blood grouping. While a patient with blood group AB was, of course, not immune to hemolytic anemia, the rarity of this group in the population and the fact that improper technic of testing blood with potent cold hemagglutinins would result in such a grouping required careful checking of such a result. Grouping and compatibility tests by the slide technic may be adequate for most blood transfusions, but in severe anemias, especially when there is a possibility of potent cold hemagglutinins, the tests should be performed in tubes at 37 C. with red blood cells completely freed of plasma by repeated washings with warmed isotonic salt solution.

The tables (2 and 3) which follow summarize and amplify the above considerations.

Hemolytic Anemia and Hemoglobinuria Associated with Cold Hemagglutinins

Hemolytic anemia associated with high titers of cold hemagglutinins has, in our experience, been one of the common forms of hemoglobinuria. As noted in Table 4, some of the cases occurred with pneumonia (H. M., V. A., R. W., M. S.) while in others there was no related pathologic condition (J. S., S. Y.). The former cases were all acute, the cold hemagglutinins were of high titer for a short time and after blood transfusions and recovery from the acute anemia, the patients remained well. The only exception to the latter statement was the case of R. W. who had two hemoglobinuric episodes separated by an interval of six months. At the time of the first episode, the evidence of pneumonia was equivocal and the cold hemagglutinin titer was 1:320; at the time of the second,

pneumonia was present and the titer was 1:2560. These patients had primary atypical pneumonia, presumably of viral origin; therapy consisted of blood transfusions.

The other cases form a heterogeneous group, one case being acute, like the pneumonia cases, and the other recurrent but without anemia. The main

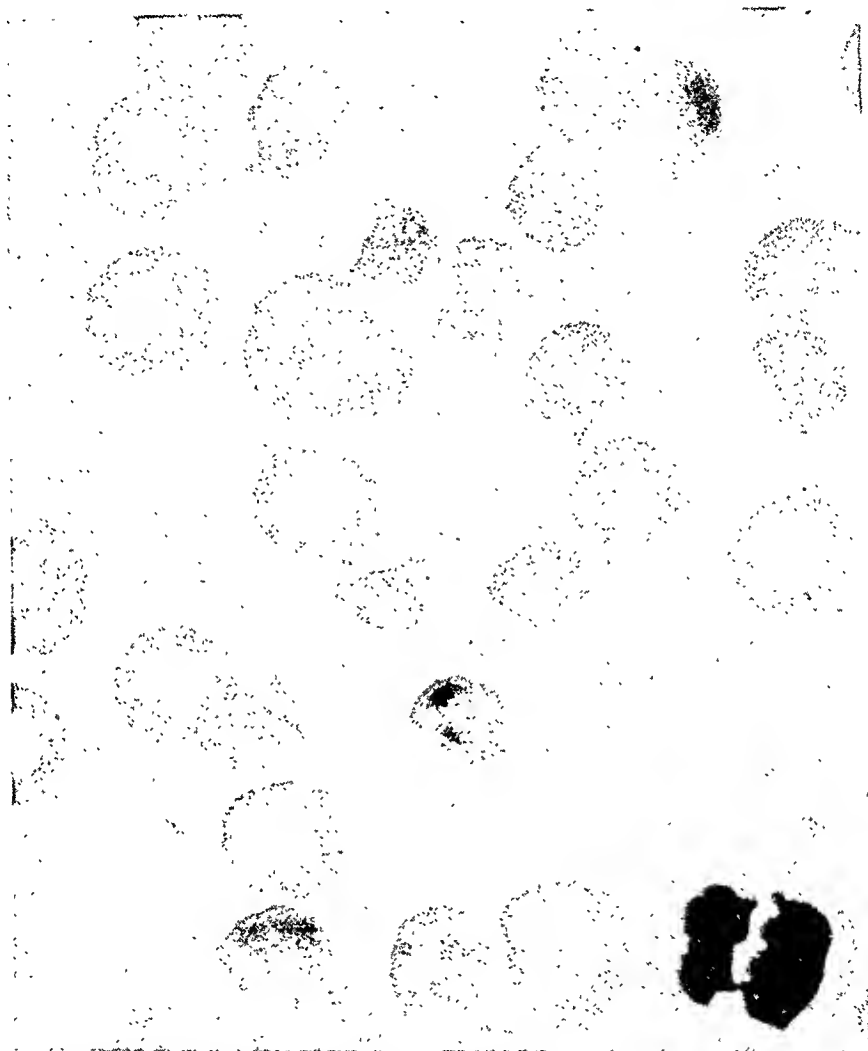


FIG. 2. Fragmentation of red cells in a case of hemolytic anemia following treatment with sulfapyridine.

clinical manifestations in the second case were peripheral gangrene, Raynard's syndrome and hemoglobinuria. In addition to these we have seen a number of cases of chronic hemolytic anemia of unknown causation without hemoglobinuria in which a high titer of cold hemagglutinins was present. In these, examination of the blood plasma for hemoglobin pigments was not made. These cases were strikingly different from the group with hemoglobinuria in the following respects: (1) The anemia was of long duration; (2) there were no acute exacerbations of the hemolytic process once it was initiated; (3) some cases had spher-

TABLE 2
DIAGNOSIS OF HEMOGLOBINURIA

POSITIVE FINDING	DIAGNOSIS
Fragmentation of erythrocytes	Drug idiosyncrasy
Heinz bodies	Phenylhydrazine or sulfonamide intoxication
Plasmodium falciparum	Blackwater fever
Bartonella	Oroya fever
Anti-Rh agglutinins and blocking antibodies	Incompatible transfusion
Donath-Landsteiner test and Wassermann test	Syphilitic paroxysmal cold hemoglobinuria
Cold hemagglutinin test and cold hemolysis with shaking test	Paroxysmal cold hemoglobinuria (primary atypical virus pneumonia and others)
Acid hemolysis test heat resistance test and hemosiderinuria	Marchiafava-Micheli Syndrome
Atypical agglutinins and hemolysins	Acquired hemolytic anemia
Hemagglutination in bovine albumin and with anti-human serum-rabbit serum	Significance not established
Absence of hemoglobinemia	Myoglobinuria
Positive blood culture	Sepsis
Precipitated by environmental cold	Cold hemagglutinin and syphilitic paroxysmal cold hemoglobinuria
Precipitated by exercise	Physiologic(?) and march hemoglobinuria

TABLE 3
SPECIAL STUDIES IN CASES OF HEMOGLOBINURIA

Blood spread examination	Fragmentation of red cells, spherocytes Autoagglutination, erythrophagocytosis Malarial plasmodia, bartonella Heinz bodies
Spectroscopic examination of blood plasma	Alpha absorption maximums oxyhemoglobin 576 methemoglobin 630 methemalbumin 624 sulfhemoglobin 620 Schumm test 558
Spectroscopic examination of urine	Alpha absorption maximums myoglobin 581 metmyoglobin 636 oxyhemoglobin methemoglobin
Hypotonic saline fragility of erythrocytes	Increased in burns, drug idiosyncrasy, incompatible transfusions, idiopathic hemolytic anemia, sepsis, spherocytosis
Urine examination for hemosiderin	Perpetually positive in Marchiafava-Micheli syndrome Usually negative in all other types
Immunologic study	Syphilitic and nonsyphilitic paroxysmal cold hemoglobinuria, Marchiafava-Micheli disease, incompatible blood transfusions, idiopathic hemolytic anemia

cytic red blood cells; (4) splenectomy cured the anemia, but at times the response was slow and the cold hemagglutinins persisted for varying periods. Further observations are necessary to establish the relationship between these cases and the others mentioned before. For comparison the non-hemoglobinemic cases (Y. K., L. K., N. M.) are included in the table.

TABLE 4
HEMOLYTIC ANEMIA ASSOCIATED WITH COLD HEMAGGLUTININS

PATIENT AND AGE	DURATION OF HEMOLYTIC DISEASE	THERAPY	ASSOCIATED FACTORS	HEMOGLOBINURIA	SEVERITY ANEMIA	HIGHEST TITER	DURATION HIGH TITER	CAN-GRENE	RECURRENCES
H. M. 32	3 days	Transfusions	Pneumonia	1 day	4+	1:1280	1-2 days	+	None
V. A. 17	2 days	Transfusions	Pneumonia; sulfadiazine	Absent	4+	1:10,000	3-5 days	0	None
R. W. 2	3 days	Transfusions	Pneumonia	3 days	4+	1:2560	1 day	0	One
M. S. 61	<2 weeks	Transfusions	Dinitrophenol; pneumonia	Not examined	4+	>1:3000	Not determined	0	None
Y. K. 26	3 months	Splenectomy	Pregnancy	Absent	4+	1:1600	2 months	0	None
L. K. 70	3 years?	Protection from cold, splenectomy	None	Absent	4+	>1:4000	>3 months after splenectomy	0	None
N. M. 24	2 months	Splenectomy	None	Not examined	0	High	Not determined	0	None
J. S. 18	2 days	Transfusions	None	2 days	4+	Not done	1 day	0	None
S. Y. 60	15 years	Protection from cold	None	Few hours	0	1:20,000	Many years	+	Many

As we have pointed out in a previous communication, we have observed patients convalescent from primary atypical pneumonia with high titers of cold hemagglutinins, but without hemolytic anemia or any clinical disturbance directly related to the cold hemagglutinins.⁷ In one case (V. A.) of hemolytic anemia after pneumonia, but without hemoglobinuria or hemoglobinemia (the Schumm test was not used in this case), we were able to produce intravascular hemolysis by immersing a finger in ice water.

REPORT OF CASES

Case 1

H. M., a 34 year old white female patient was admitted to another hospital. Prior to her present illness she had been entirely well. Two weeks before she developed a respiratory infection with nasal stuffiness, mild sore throat and low grade fever. After several days this progressed to a cough productive of small amounts of mucopurulent sputum which was neither bloody nor foul. She continued to run an irregular fever as high as 103 F. and was treated symptomatically with aspirin. Two days before admission she was observed to be quite pale; the next morning she observed that her urine had the color of port wine. On the day of admission the urine was normal in color; she fainted once because of weakness and observed tingling of her toes. Very low environmental temperature was present on this day. En route to the hospital by ambulance she noted coldness and numbness in both feet.

On examination she was irrational, extremely pale but not icteric. The respiratory rate was 36 per minute, and she coughed frequently. The rectal temperature was 104 F. The positive findings consisted of moderate dullness with bronchovesicular breath sounds and many scattered medium rales over both lower lobes of the lungs. The heart rhythm was regular, the rate 130 per minute. An apical systolic murmur was heard. Neither the liver nor the spleen was palpable. Both feet, but especially the left, were cool about the ankles and cold about the toes. They were dead white in color and, on the left, insensitive to painful stimuli. The peripheral arterial pulses in the feet were not examined at this time. The day after admission strong posterior tibial and normal popliteal pulses were felt in both legs as well as a good dorsalis pedis pulse on the right. The left dorsalis pedis could not be felt, but this may have been due to edema in this region which was quite marked on the second day. Laboratory studies on the following day (Table 5) showed the white blood count to be 19,100 with neutrophils nonsegmented 23 per cent, neutrophils segmented 59 per cent, lymphocytes 12 per cent, monocytes 1 per cent, myeloblasts 1 per cent, myelocytes 4 per cent, normoblasts 6 per 100 white blood cells. The icterus index was 2, bilirubin 0.4 mg. per 100 ml., direct van den Bergh reaction negative, total serum proteins 5.9 Gm. per 100 ml., serum cholesterol 177 mg., alkaline phosphatase 11 King Armstrong units per 100 ml., thymol turbidity test 2 plus and cephalin flocculation test 3 plus. The heat resistance test of the erythrocytes was negative. Agglutination tests using the patient's erythrocytes and dilutions of serum revealed a titer of less than 1:5 at 37 C., 1:20 at 23 C. and 1:1280 at 6 C. The cold hemolysis with shaking test (see below) was positive. Immune type of hemolysis were not demonstrable with added complement at 37 C. X-ray films of the chest revealed patchy areas of consolidation in each lower lobe. Pathogenic micro-organisms could not be isolated from the sputum.

The diagnoses on admission were bilateral lower lobe pneumonia, possibly of viral origin, severe anemia and impending gangrene of the feet, more marked on the left. Because of the incorrect deduction that occlusion of a major artery to the extremities might be present, the left foot was packed in ice. This was continued for six hours and, when the pack was removed, incipient gangrene of all the toes and part of the foot on the left and the tips of several toes on the right was obvious. Marked edema of the dorsum of the left foot became evident in the region of the junction between the viable and nonviable portions. The details concerning the subsequent fate of the feet and the various measures used to effect separation of the sloughs and final successful skin grafting do not concern us here.

The severe anemia was treated by transfusions, a total of 2000 ml. of blood having been administered. There were no reactions to this therapy and, as indicated in the table, rapid improvement in the hematologic findings ensued. There was considerable difficulty in the initial attempt to group this patient's blood. The laboratory first reported group AB and later group B but, even before the correct grouping (O) was ascertained, a transfusion had been given. Fortunately, only group O blood was available; all the blood given was of this group.

The subsequent clinical course was stormy for the first week, dominated by the severe pneumonia and gangrene of the feet. High irregular fever persisted during this period. After the end of the second week there was progressive general improvement and acral mummification as mentioned before.

TABLE 5
COLD HEMAGGLUTININ HEMOLYTIC ANEMIA WITH GANGRENE OF TOES

DATE	HB.	RBC	RETICU- LOCYTES	HEMA- TOCRIT	HEMOGLOBIN PIGMENTS IN PLASMA	COLD HEM- AGGLU- TININ	HYPOTONIC SALINE FRAGILITY	OTHER DATA
	per cent	million	per cent		mg. per 100 ml.	titer	per cent	
Admission*	33	1.35	11	16	71.4 oxyhemo- globin trace methemalbu- min (2+)	1:1280	0.52 to 0.24	Spherocytes, bone mar- row 80 per cent, nor- moerythro- blasts, he- moglobinu- ria absent
2 days later*	46	2.35	21	21	10.9 methemal- bumin	1:800	0.48 to 0.20	Hemosidcri- nuria ab- sent, acid hemolysis test nega- tive, occa- sional spherocyte pres- ent
5 days later*	53	2.83	8	27	3.2 (normal)	1:400	0.48 to 0.20	Spherocytes absent, Kahn test negative, Donath- Landsteiner test nega- tive
12 days later	64	3.00	2		3.3 (normal)	1:800		Sedimenta- tion rate 84 mm. in 1 hour
42 days later	80	4.17	0.5	39		1:200		

* 500 ml. blood transfusion before blood count.

In addition to the tests given above, blood obtained from this patient on the first day of observation was readily hemolyzed when shaken in the cold. We have previously elucidated the details of this cold hemolysis with shaking test and have indicated its fundamental difference from the Donath-Landsteiner reaction.⁷ We believe that the marked sensitivity of strongly cold-agglutinated erythrocytes to mechanical trauma explains, at least in part, the mechanism

by which cold hemagglutinins may induce a hemolytic syndrome. Studies in other cases have demonstrated that immersion in ice water of an extremity of a patient with potent cold hemagglutinins leads to hemolysis of blood in the chilled part.⁶ The amount of hemoglobin liberated is large considering the volume flow in such an experiment, but when viewed from the general body economy it is quite low, often not enough to cause hemoglobinuria. This may explain the failure of the chilling of the foot to cause further exacerbation of the anemia and a recurrence of hemoglobinuria.

Other evidence of the strictly mechanical nature of the hemolysis under such circumstances is afforded by the observation of hemolysis following the circulation of blood with potent cold hemagglutinins through capillary tubing immersed in ice water. A 3 foot length of capillary tubing was arranged in such a fashion that blood could be injected by means of a syringe into and through it from one end and received at the other, while the tube was immersed in water

TABLE 6
HEMOLYSIS RESULTING FROM THE INJECTION OF BLOOD CONTAINING POTENT
COLD HEMAGGLUTININS THROUGH COLD CAPILLARY TUBING

SPECIMEN	MG. HEMOGLOBIN PER 100 ML. PLASMA
Blood 4 C., no wait	0
Blood 4 C., $\frac{1}{2}$ minute wait	139
Blood 4 C., 2 minute wait	340
Blood 4 C., 5 minute wait	391
Blood 37 C., 2 minute wait	17
Blood remaining in syringe	64

NOTE: Blood was injected into the tubing at 4 C. or 37 C. and left there for the indicated waiting period after which, by further injection, it was forced through and collected.

of any desired temperature. When this was carried out at 20 C. or 37 C. with oxalated blood containing potent cold hemagglutinins, hemolysis did not result. When, however, the same blood was injected through the capillary previously immersed in ice water, marked hemoglobinemia resulted. In Table 6, the results of such an experiment, in which one large sample of blood was used, are given.

We believe that this patient had primary atypical pneumonia. The development of a high titer of cold hemagglutinins is a frequent occurrence in this condition. The anemia resulted from the increased mechanical fragility of the strongly cold-agglutinated red cells and subsequent intravascular hemolysis. The gangrene was initiated by environmental cold which caused agglutination of the erythrocytes in the small blood vessels and was greatly aggravated by the use of an ice pack.

The Marchiafava-Micheli Syndrome of Paroxysmal Nocturnal Hemoglobinemia

We have observed 8 cases of the Marchiafava-Micheli syndrome of paroxysmal nocturnal hemoglobinuria and, in general, our observations were identical with

those reported by others. We have been impressed by one finding that is not included in the usual description of this disease, namely, the frequency of biliary calculi. Five of the 8 cases we examined had this disorder and in two, gall

TABLE 7
FINDINGS IN 6 PATIENTS WITH MARCHIAFAVA-MICHELI SYNDROME

AGE AT ONSET	DURATION	PRESENTING SYMPTOM	BLOOD FINDINGS*	MEAN CORPUSCULAR VOLUME	ACID HEMOLYSIS TEST, HEAT RESISTANCE TEST, PERFETUAL HEMOSIDERINURIA	HEMOGLOBINURIA	COMPLICATIONS
24	years 21	Dark urine	Hb. 32 to 70% Retic. 3 to 8% WBC 4000	100	Positive	Occasional	Cholelithiasis, cerebral thrombosis(?) slight icterus
21	21	Renal pain	Hb. 34 to 75% Retic. 0.5 to 9% WBC 7000	90	Positive	Rare	Cholelithiasis, slight icterus
26	1½	Weakness, dark urine	Hb. 55% Retic. 17% WBC 4500	119	Positive	Frequent	Cholelithiasis, thrombocytopenia, marked icterus
50	10	Weakness, dark urine	Hb. 51 to 69% Retic. 1 to 7% WBC 4500	106	Positive	Rare	Cholelithiasis, thrombocytopenia, angina pectoris, slight icterus
25	8	Weakness	Hb. 20 to 46% Retic. 0.5 to 28% WBC 5000	90	Positive	Frequent	Thrombocytopenia, marked icterus
47	2	Dark urine	Hb. 55% Retic. 8% WBC 4800	113	Positive	Frequent	Thrombocytopenia, marked icterus

* Hb. indicates hemoglobin; Retic., reticulocytes; WBC, leukocyte count.

bladder operations were necessary. The findings in 6 of these cases are summarized in Table 7.

Case 2

In the presentation of the data in this case we shall emphasize material that has not been published previously and merely indicate some of the other facts that have been described on previous occasions by others.

This patient was first studied nine years ago because of pallor, weakness, paresthesias, ankle edema, occasional fever and dark urine. On examination she was pallid and icteric. The thyroid gland was moderately enlarged and nodular. The spleen was hard and was felt 4 cm. below the left costal margin. Purpuric spots, ecchymoses and petechiae were scattered over the body. A thrombosed hemorrhoid was present. The hemoglobin was 65 per cent, the red blood cell count 2,340,000, the white blood cells 3,100 and the platelets 90,000. The hematocrit was 27.2 per cent and the reticulocytes 3 per cent. A Price-Jones curve showed the average diameter of the erythrocytes to be 8 microns (μ). The bone marrow was of normal cellularity with increase in megakaryocytes, and the normo-erythroblasts numbered 62 per cent. The coagulation time of venous blood was 5 minutes, the bleeding time (Duke) 10 minutes; the tourniquet test was positive, and the clot retraction was good. Free hydrochloric acid was present in the gastric contents. The Wassermann and Kahn tests fluctuated spontaneously between negative and moderately positive. The kidney function was normal, but the urinalysis showed traces of albumin. Over a period of 38 days every specimen of urine passed was examined for hemosiderin and hemoglobin. The latter was consistently negative while 70 per cent of the tests for hemosiderin were positive in varying degrees. At another time 12-hour pooled urine specimens over a 28-day period were examined. Again hemoglobin could not be detected whereas every specimen but one showed hemosiderin. While there was no obvious periodicity to the excretion of hemosiderin, 33 per cent of the specimens passed between midnight and 7 a.m. were markedly positive and 8 per cent negative; whereas, over the period of noon to 6 p.m., 9 per cent were markedly positive and 33 per cent were negative. Over this entire period of 68 days there was not a single day in which hemosiderin was absent from every urine. (In the other patients with this disease, hemosiderinuria was found more frequently than in this patient.) Quantitative study of the urinary urobilinogen excretion on many occasions revealed a marked variation from 0.3 mg. to 10 mg. in 24 hours. Stool examinations were occasionally normal in this regard, but on several occasions excretions as high as 433 mg. and 780 mg. per 24 hours were found. Over a 10-day period specimens of blood plasma were obtained at 8 a.m. and 8 p.m., and quantitative estimations of the total hemoglobin pigments were performed. In these tests there was not a single exception to the rule that the pigment level was considerably higher in the morning specimens. On one occasion the morning level was 50 times higher than the evening and the highest value recorded was 280 mg. per 100 ml. Spectrophotometric analysis of a small series of these specimens of plasma showed a constant level of methemalbumin and a fluctuation in the oxyhemoglobin concentration with higher readings in the morning.

This patient is still under observation and, as is usual in such patients, has not improved on various therapeutic regimes. Six blood transfusions were administered; two were uneventful, one was followed by hemoglobinuria and the others by chilly sensations and fever. Recently, she has developed mild diabetes mellitus.

Acute Hemolytic Anemia and Hemoglobinuria Following Ingestion of Drugs

In Table 8 we have listed only cases with unquestioned hemoglobinuria following the ingestion of drugs. Excluded are similar cases in which observations were not complete enough to prove the presence of hemoglobinuria.

Case 3

D. G., a 43 year old Italian chef, was admitted to the hospital with a history of polycythemia vera for the previous two years, treated by occasional phlebotomies. Two weeks before admission he started to take phenylhydrazine and shortly thereafter noted the onset of progressive weakness, pallor and easy fatigability. The amount of drug that was ingested could not be determined. For several days he had noted a port-wine color of the urine, but this stopped three days before he came under our care.

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On examination he was pale, and his temperature was 101 F. The spleen was hard, its lower edge being 6 cm. below the left costal margin. The liver was palpable 2 cm. below the right costal margin.

TABLE 8
HEMOGLOBINURIA FOLLOWING INGESTION OF DRUGS

PATIENT AND AGE	UNDERLYING DISEASE	DRUG	ONSET hr.	BLOOD FINDINGS*	BLOOD TRANSFUSIONS ml.	OTHER DATA†
V.B., 42	Tonsillitis	Sulfanilamide 6 Gm.	36	Hb. 45%, Retic. 5.2%, Fragility 0.44 to 0.24%	1500	Urea N 48 mg. per 100 ml., I.I. 32, hemoglobinuria lasted 24 hr.
L.K., 2	Pharyngitis	Sulfadiazine 3 Gm.	48	Hb. 34%, Fragility 0.44 to 0.28%	325	Rapid recovery, spleen palpable 1 fingerbreadth
E.L., 36	Tonsillitis	Sulfanilamide 2½ Gm.	48	Hb. 42%, Retic. 0.5%	2500	Urea N 170 mg., azotemia for 2½ wk., I.I. 15, anuria 24 hr.
B.E., 39	Furuncle in nose, bronchopneumonia	Sulfapyridine 11 Gm.	36	Hb. 39%, rose to 67%	1500	Urea N 69 mg., CO ₂ 33 vol. per 100 ml., spleen palpable 4 fingerbreadths, died in 2 days in collapse
P.S., 7	Sinusitis	Sulfanilamide	72	Hb. 20%, rose to 45%	1200	Hb. fell 25% in 24 hr. despite 650 ml. transfusion, spleen palpable 2 fingerbreadths
J.M., 30	Erysipelas	Sulfanilamide 12 Gm.	48	Hb. fell from 104% to 34% in 2 days, Retic. 8%	2500	No azotemia, I.I. 108, hemoglobinuria lasted 24 hr., stool urobilinogen 798 mg. per 24 hr.
D.G., 47	Polycythemia	Phenylhydrazine	?	Hb. 46%, Retic. 28%, Fragility 0.56 to 0.16%	1500	No azotemia, Heinz bodies present, thrombocytosis, Schumm test positive

* Hb. indicates hemoglobin; Retic., reticulocytes; Fragility, fragility of red blood cells.
† I.I. indicates icterus index.

The blood findings were as follows: hemoglobin, 46 per cent; erythrocyte count, 2,500,000; leukocyte count, 21,100; platelet count, 950,000. The hematocrit was 24 per cent, and the reticulocytes numbered 28 per cent. The differential count showed nonsegmented neutro-

phils, 17 per cent; segmented neutrophils, 61 per cent; segmented eosinophils, 3 per cent; lymphocytes, 14 per cent; monocytes, 2 per cent; myelocytes, 3 per cent and 3 normoblasts per 100 leukocytes. Spherocytes were present. In addition to the spherocytes, which were well demonstrated in the wet preparation of fresh unstained blood, many Heinz bodies were apparent. These appeared as refractile bodies the size of platelets overlying the periphery of the erythrocytes. There was no sickling of the red cells. The marrow obtained by aspiration of the sternum was richly cellular showing many megakaryocytes and an increase in the normo-erythroblasts to 50 per cent.

Spectroscopic examination of the plasma revealed minimal absorption at 576μ , indicative of small amounts of oxyhemoglobin (probably within the limits of the error in the method). There was no absorption in the region of 625μ but, upon the addition of concentrated ammonium sulphide (Schumm test), a distinct band became visible at 558μ . This latter finding was again positive three days later, after which it was no longer present. Six urine specimens failed to reveal hemoglobin or hemosiderin, and the urinary output was normal. Kidney function tests showed no abnormalities.

Serologic study failed to show any positive findings. The Wassermann and Donath-Landsteiner tests were negative. The cold hemagglutinin titer was 1:4. Hemagglutinins against homologous or heterologous erythrocytes were not demonstrable in physiologic salt solution, 30 per cent bovine albumin or human serum mediums at 25 C. and 37 C. The acid hemolysis and heat resistance tests were negative. There were no hemolysins even when adequate amounts of guinea pig complement were added to the system.

Thorough study of the blood chemistry did not reveal any findings of note. The urea nitrogen was 18 mg. per 100 ml.; the icterus index was 8; the cephalin-cholesterol flocculation was 2 plus.

The elevated temperature observed on admission returned to normal in two days. Three blood transfusions of 500 ml. each were administered without reactions. There was a progressive rise in the hemoglobin to 73 per cent and in the red blood cells to 3,730,000. The reticulocytes were markedly elevated for nine days. Spherocytosis disappeared after eight days. The platelets rose to a peak of 1,200,000 seven days after admission. Before discharge the spleen was noted to have decreased considerably in size. After complete subsidence of the hemolytic process and restitution of the blood picture to polycythemic levels, the patient was treated with radioactive phosphorus.

Acute Idiopathic Hemolytic Anemia with Hemoglobinuria

The three cases of acute idiopathic hemolytic anemia with hemoglobinuria listed in Table 9 were grouped together only because an etiologic factor could not be ascertained after detailed questioning of the patients and their relatives, examination of the patients, and thorough serologic and hematologic study of their blood. In these cases, important information may be obtained in the future by use of newer serologic techniques.

Case 4

S. D., a 5 year old boy who previously had been well, was taken ill three days before admission with restlessness. Two days before admission, he had a chill with a fever of 104 F., and his urine was noted to be very dark in color. At this time, the administration of usual doses of codeine, aspirin and phenacetin was started. Subsequently, marked pallor and icterus were observed, and he vomited several times. There was no significant family history of hematologic disorders (hematologic examination of the father, mother and one sibling was negative), and the patient had not been exposed to any known toxic agent.

Upon examination he was prostrated, acutely ill, pallid and icteric. The temperature was 100 F., the pulse rate 140 per minute and the respiratory rate 30 per minute. There were no other positive findings; neither the liver nor spleen were palpable. Some of the hematologic data are given in Table 10. On admission (Apr. 17) the red blood cell count was 1,160,000, the white blood cell count was 10,700, the platelet count was 190,000 and the

TABLE 9
ACUTE IDIOPATHIC HEMOLYTIC ANEMIA WITH HEMOGLOBINURIA

PATIENT	AGE	HB.	RBC	RETICU- LOCYTES	WBC	HEMOGLOBINEMIA MG. PER 100 ML.		HEMOGLOBINURIA MG. PER 100 ML.		BLOOD TRANS- FUSIONS	ADDITIONAL FINDINGS
						Peak	Dura- tion	Peak	Dura- tion		
		<i>per cent</i>	<i>millions</i>	<i>per cent</i>			<i>days</i>		<i>days</i>	<i>ml.</i>	
D. C.	42	31	1.42	10 to 19	38,000	532	10	722	6	2500	Diabetic ke- tosis, transient spherocy- tosis, re- covery followed by a mild relapse
L. M.	4	71	5.02		11,200	338			2	400	Transient spherocy- tosis, fragemen- tation of red cells, rapid re- covery
S. D.	5	33	1.16	1 to 6		700	9	100	5	500	Transient spherocy- tosis, slow recovery despite rapid sub- sidence of hemoly- sis

TABLE 10

	Hb.	Reticulo- cytes	Plasma Hb.	Urine Hb.	Fecal Uro- bilinogen	Urine Hemosiderin
	<i>per cent</i>	<i>per cent</i>	<i>mg. per 100 ml.</i>	<i>mg. per 100 ml.</i>	<i>mg./24 hr.</i>	
Admission*	33	1	700	—	—	0
1 day later	57	1	350	100	—	0
3 days later	68	3	102	10	171	0
4 days later	70	6	52.5	2.0	201	+
5 days later	64	5	42	0	36.5	+
7 days later	56	2	10	0	39.0	0
20 days later	65	3	0	0	2.5	0
54 days later	90	1	0	0	—	—

* 500 ml. blood transfusion.

hematocrit was 15 per cent. The nonsegmented neutrophils numbered 37 per cent; segmented neutrophils, 24 per cent; lymphocytes, 32 per cent; monocytes, 6 per cent; segmented eosinophils, 1 per cent and there were 4 erythroblasts per 100 white blood cells. Spherocytes

were present, and the hypotonic saline fragility of the erythrocytes revealed beginning hemolysis in 0.60 per cent sodium chloride solution and complete hemolysis in 0.40 per cent sodium chloride solution. Twenty-eight per cent of the red blood cells measured 6.5 microns (μ) by the Price-Jones technic. Examination of the bone marrow, obtained by aspiration of the sternum, revealed highly cellular spreads in which the normo-erythroblasts amounted to 55 per cent of the total nucleated elements. Other studies revealed negative findings in the following tests: acid hemolysis test, Donath-Landsteiner and cold hemagglutinin tests and test for hemolysins using guinea pig complement. Spectrophotometric analysis of the pigments in the plasma showed upon admission tremendous amounts of oxyhemoglobin and a much smaller amount of methemalbumin as indicated by deep bands at 540 μ and 576 μ and a lesser band at 620 μ . With regard to the latter it should be noted that the band persisted after the addition of sodium cyanide and 3 per cent hydrogen peroxide. In the plasma, studied four days later, the oxyhemoglobin bands were much less marked, whereas relatively considerable amounts of methemalbumin were still present. Pigment analysis of the washed laked erythrocytes showed only oxyhemoglobin. Study of the urine, passed at the time of admission, showed large amounts of oxyhemoglobin and a trace of methemoglobin.

Routine urinalysis revealed specific gravities as high as 1.022. There was a trace of albumin, and the centrifuged sediment was either negative or showed a few coarsely granular casts and white blood cells. The blood urea nitrogen was 77 mg. per 100 ml., icterus index 40, cholesterol 240 mg., cholesterol esters 70 mg. and total serum proteins 6.1 Gm. per 100 ml. The Wassermann test was negative. The blood culture was sterile.

The child was treated with a continuous intravenous infusion for 24 hours in addition to a transfusion of 500 ml. of blood. There was rapid clinical improvement, and gross hemoglobinuria cleared in 24 hours. The urinary output remained normal throughout. As observed in the appended chart, three weeks after admission the red blood count was only 2,800,000 per cu. mm. despite the fact that excessive hemolysis had stopped four days after admission. It is also to be noted that throughout the course reticulocytosis was low or normal. As far as could be determined anemia was continuing in the face of a morphologically active marrow and a normal degree of hemolysis. When the patient was examined again four weeks later the blood count was normal. There has not been any recurrence of a hemolytic process in five years.

SUMMARY

1. A large clinical experience with cases of hemolytic anemia with hemoglobinuria revealed four categories, namely, the Marchiafava-Micheli syndrome of paroxysmal nocturnal hemoglobinuria, cases caused by cold hemagglutinins, or by drugs, and an idiopathic variety. These were the only types considered in this communication.

2. The importance of spectroscopy of the plasma and the urine was mentioned, and the main characteristics and differentiation of the absorption bands for the common hemoglobin derivatives were given. Exclusive of chronic cases (mainly the Marchiafava-Micheli syndrome), hemoglobinuria was of short duration.

3. The importance of the detection of persistent hemosiderinuria in the diagnosis of the Marchiafava-Micheli syndrome and the generally unexplored hemosiderinuria of the other hemoglobinurias were discussed. The relatively infrequent occurrence of serious impairment of renal function, especially in the chronic hemoglobinurias, was pointed out.

4. We have indicated the battery of serologic tests which should be performed in cases of hemoglobinuria in order to arrive at an accurate diagnosis.

5. Our findings in the morphologic examination of the peripheral blood and bone marrow were presented.
6. When anemia supervened, large and frequent blood transfusions were required. In view of the short course of most of the acute cases, vigorous blood replacement when blood destruction was rapid, effected a cure in a large percentage of cases. We believe that splenectomy is contraindicated in the hemoglobinemias or hemoglobinurias. In view of the reactions which followed blood transfusions in the Marchiafava-Micheli syndrome, a minimal number of transfusions should be given in such cases.
7. A detailed case report illustrating each of the four main types and a tabulation of the clinical data of the other cases that we have observed were appended.

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AMYLOID "TUMORS" OF THE LARYNX, TRACHEA AND BRONCHI

A HISTOLOGIC STUDY OF FIFTEEN CASES*

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The present study has been limited to "tumor"-forming amyloid present in the region of the larynx, trachea or bronchi.

Burrow and Neumann² reported the first case of amyloid "tumor" of the larynx in 1875. A number of case reports of amyloid tumor involving the larynx, trachea or bronchi have been published since that time. The pertinent literature has been reviewed by Pollak,^{10, 11} New,⁹ Schmidt,¹⁵ Kramer and Som,⁷ and Rey.¹³

CLASSIFICATION AND DESCRIPTION

According to New, these amyloid tumors may be considered to occur either in association with amyloid degeneration elsewhere in the body, or as isolated deposits. The isolated deposits may be divided into three groups: (1) the diffuse subepithelial infiltration of amyloid, (2) the tumor-forming amyloid deposit, and (3) amyloid degeneration in a pre-existing tumor.

Gross appearance. The typical gross description of the amyloid tumor has been that of a waxy, translucent, yellow or yellow-gray swelling without ulceration of the overlying mucosa. The lesion has been described as having either a smooth or a nodular outline and as being either diffuse or well localized. The tissue has had a hard consistency¹² on palpation and has been difficult to curet because of this hardness; or, it has been friable and vascular, in which case the color has been red.

Microscopic appearance. There is no difference in the microscopic appearance of the diffuse and the tumor-forming type of lesion.¹³ There is a subepithelial deposition of the homogeneous hyaline amyloid material. The mucosa and submucosa are elevated by the amyloid deposit which is seen in layers closely packed between the connective tissue. The walls of the blood vessels are especially affected.⁹ Beavis¹ noted that the rounded masses present in many amyloid tumors appeared to arise in the blood vessels. Many authors noted that the basement membrane of the mucous glands was swollen by the homogeneous amyloid.¹⁴ Courvoisier⁴ described a lesion in which this involvement had progressed so that the glands had become replaced by masses of amyloid material. The presence of giant cells has been considered by some to be typical of the lesion.¹³

Typically, the amyloid material is stained selectively by the Congo red stain

* Abridgment of a portion of thesis submitted by Dr. Stark to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Surgery. Received for publication, July 6, 1948.

and iodine, and it is stained metachromatically with crystal violet and other related aniline dyes. Beavis and others have described lesions in which apparently only the oldest portions of the deposit stained as amyloid, the remainder staining poorly. Many authors⁶ consider that these specific color reactions may be weak or not present at all.^{3, 5}

MATERIAL AND METHOD OF STUDY

Data on 28 cases with the diagnosis of amyloid degeneration of the larynx, trachea or bronchi were found in the surgical files of the Mayo Clinic. These cases were included in the present study. In all of the cases the diagnosis of amyloid disease had been based on microscopic examination of tissue removed for biopsy or at operation. In the vast majority of instances, the diagnosis had been based on the appearance of an eosinophilic homogeneous hyaline material seen in sections stained with hematoxylin and eosin. Adequate amounts of tissue for further study with special stains were available in 24 cases. In three cases only microscopic sections stained with hematoxylin and eosin were available. In one case (New,⁹ Case 2) neither slide nor tissue was available.

All of the tissue used in this study had been preserved in 10 per cent formalin. In 8 cases enough tissue was available to permit the staining of a frozen section for fat using Sudan III. The tissues were embedded in paraffin. Seven serial sections, each approximately 7 microns in thickness, were made from each paraffin block. These were stained with hematoxylin and eosin, van Gieson's stain and the so-called specific amyloid stains: crystal violet, methyl green, Congo red and iodine. In order to check the accuracy of these special stains, sections from an atrophic ovary and a "sago" spleen were used as controls on each slide.

Technics of Staining with Special Stains

The staining methods used were modifications of standard procedures.⁸

Crystal violet stain:

1. Stain the section in a 0.5 per cent solution of crystal violet for five minutes.
2. Wash in a 1 per cent aqueous solution of acetic acid.
3. *N. B.* Wash thoroughly in water to remove all traces of acid.
4. Mount in levulose. Seal the cover slip with Duco cement.

Methyl green stain:

1. Stain the section in a 0.33 per cent aqueous solution of methyl green for twenty-four hours.
2. Wash in water.
3. Mount in water. Seal the cover slip with Duco cement.

Congo red stain:

1. Stain the section in a 1 per cent aqueous solution of Congo red for fifteen minutes.
2. Dip in a saturated aqueous solution of lithium carbonate for fifteen seconds.
3. *N. B.* Decolorize in 80 per cent alcohol until the control normal tissue contains no gross color.
4. Wash in water for fifteen minutes.
5. Counterstain with Harris' hematoxylin for one minute.
6. Wash in water for three minutes.

7. Dehydrate in 95 per cent alcohol and absolute alcohol.

8. Clear in xylol and mount in balsam.

Iodine stain:

1. Dip the section in a solution of Gram's iodine for fifteen seconds.

2. Dip in a 20 per cent solution of sulfuric acid for twenty seconds.

3. Wash in water.

4. Mount in water. Seal the cover slip with Duco cement.

The sections were studied and criteria for the microscopic diagnosis of amyloid tumor were established. Fifteen cases (Table 1) were found to fulfill these criteria. Sections from 2 of these 15 cases and from 7 selected cases not considered amyloid were stained with Mallory's phosphotungstic acid stain for further study.

PATHOLOGIC STUDY

Pathologic histology. The following descriptions are based on the appearance of sections of the amyloid tumors stained with hematoxylin and eosin unless otherwise specifically stated. Many of the specimens, being mere scraps of tissue, served only to provide a diagnosis and did not permit further histologic study. Amyloid was found to be a relatively homogeneous material which stained a varying shade of pink with eosin. This material was present in the tumor in two distinct forms, the "flake" and the "concentric layered mass". The flakes (Fig. 1) were angular, sharply outlined bits of material separated by clear artefacts. Study of a fresh frozen section of an amyloid tumor stained with polychrome methylene blue disclosed these flakes to be stained deeply blue and to be separated from each other. The material, then, tended to fragment, and the procedure of fixing and sectioning demonstrated this tendency in the form of the clear artefacts. The oval or rounded masses (Fig. 2) appeared as layered rings about a central point. Variation in intensity of staining of these rings was noted in the hematoxylin and eosin section. The metachromatic reaction of the crystal violet and methyl green stains tended to emphasize these layers (Fig. 3). In the areas in which the entire normal structure had not been obliterated by the amyloid deposit, one could see an eosinophilic homogeneous substance immediately outside of the cuboidal epithelium of the mucous glands. This same material stained as amyloid with the crystal violet stain. In areas, the cuboidal epithelium appeared compressed by a thicker ring of amyloid. Intermediate stages between this and the solid mass of concentric rings were present (Figs. 4 and 5).

Although the walls of some of the vessels contained amyloid, there was little evidence to suggest that the concentric ringed masses had developed in relation to the blood vessels. Frequently, the vessels in the region of the deposit of amyloid consisted of a space lined with endothelium which was in immediate contact with the homogeneous amyloid flakes. There was no remnant of a thicker vessel wall. Those vessels in parts of the sections that were not the site of deposit of amyloid appeared normal and were not involved in the amyloid change.

The cellular reaction at the site of deposition of amyloid was not at all char-



FIG. 1. Sharply outlined fragmented "flakes". Hematoxylin and eosin. $\times 350$

FIG. 2. Concentric ringed mass. The central defect is lined with the debris of the lining cells of a mucous gland. Crystal violet. $\times 300$.

FIG. 3. The variation in the intensity of staining reaction is well displayed in this "concentric layered mass" stained with crystal violet. Crystal violet. $\times 435$.

FIG. 4. Amyloid material deposited along the basement membrane of a mucous gland. Crystal violet. $\times 400$.

acteristic. Foreign-body giant cells were sometimes present at the periphery of a flake or mass of the substance, but this was by no means the rule. More frequently, there was a slight to moderate infiltration of small round inflammatory cells, lymphocytes and particularly plasma cells. Deposits of calcium and a small region of osteoid tissue were present in one instance (Case 10).

In those few sections which cut across epithelium, the amyloid material was noted to be present immediately subjacent to the epithelium or to be separated from the epithelium by a varying thickness of hyalinized connective tissue.

Van Gieson's stain was very helpful in the study of the histology of the sections, because the hyalinized collagen fibers (which might have been mistaken for amyloid material in the section stained with hematoxylin and eosin) were stained red, whereas the amyloid was stained a dirty yellow color.

Four sections of amyloid-bearing tissues which were stained with Sudan III were studied in an attempt to determine if lipids were present in association with the amyloid. Although there were a few globules of red-stained material between the fragments of amyloid, the greater part of the color was present in the pattern of the flakes and masses of amyloid. The color was present as pink specks so indistinct as to be visible only with high-power magnification. These specks were spaced so as to give a fairly uniform pink or in some areas red color to the fragments of amyloid. As might be expected, this staining reaction was absent in a paraffin section. The stained substance, then, was soluble in fat solvents. Four sections of specimens from lesions which proved not to be amyloid did not stain to the same degree with the Sudan III.

Special Stains

In order to establish some basis for the comparison of the reactions of the various stains with the amyloid material, the color reactions were graded on the basis of 1 to 4, 1 being the minimal color reaction which would indicate the presence of amyloid and 4 being an intense "typical" reaction. This grading was based on the tinctorial reaction present and not on the quantity of the material which presented the reaction.

Crystal violet. When the section presented areas with a dark purple hue rather than the true blue which the normal tissue stained, the reaction was graded 1. When the purple was associated with a redder violet, the grade was 2. When there were also some streaks of ruby red, the grade was 3; and when a ruby red color was present in masses rather than just streaked through tissue stained purple or violet, the grade was 4.

Methyl green. The staining reactions were similar to, but not as intense as, the reactions with crystal violet.

Congo red. A faint pink was considered grade 1 only if the control ovary remained unstained. A deep pink or red was considered a grade 4 reaction.

Iodine. A grade 1 reaction was of doubtful significance. It was a reddish hue which tended to fade completely after a day or two. Grade 2 signified a dirt-brown color which tended to fade to a distinctive khaki or a mahogany color. Grade 3 signified a dark brown or mahogany color with a suggestion of

green. Grade 4 signified a brown or mahogany stain with definite streaks of green or green-blue.

There were some points of importance which were considered when the tincorial reactions of the so-called amyloid stains were interpreted. (1) When sections stained with crystal violet or methyl green were studied, the source light was filtered to a definite blue color because white light caused a pink color to be apparent in the sections which might have been incorrectly interpreted as a

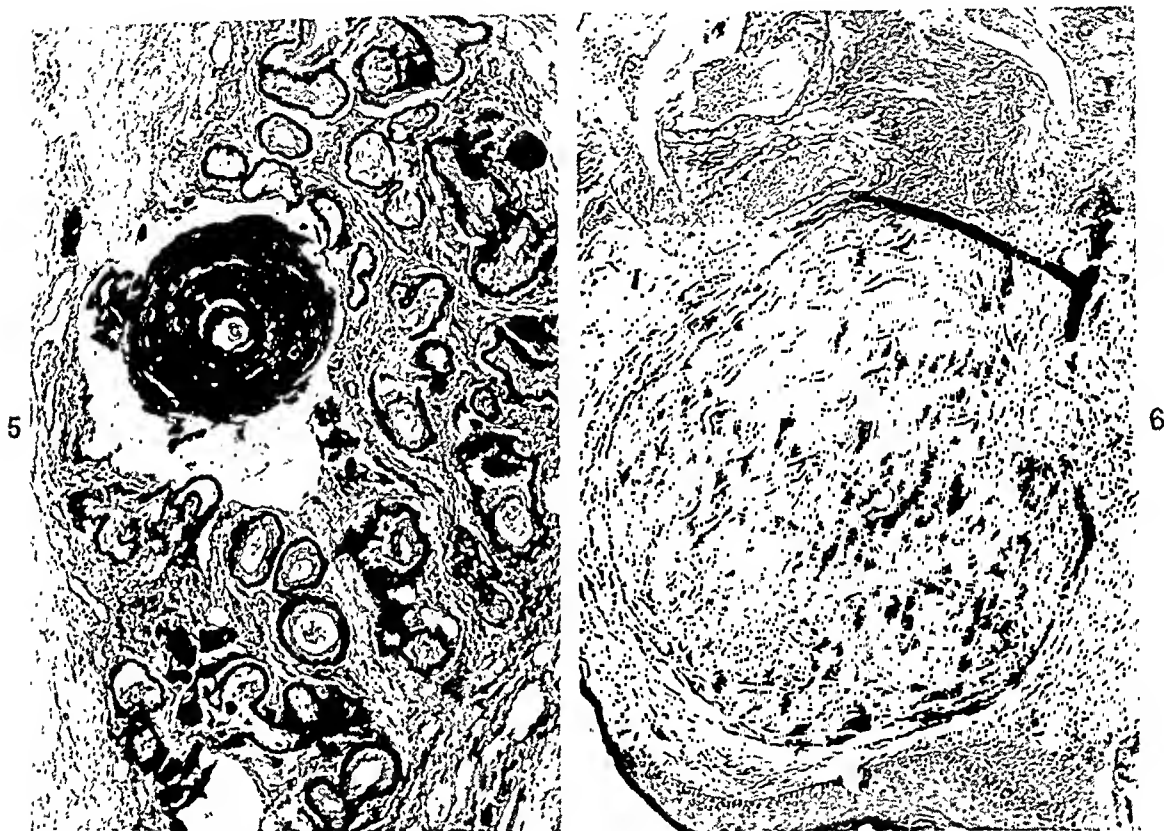


FIG. 5. A group of mucous glands with amyloid material deposited along the basement membrane. In one concentric layered mass the deposition of amyloid has progressed to almost complete obliteration of the gland. Crystal violet. $\times 85$.

FIG. 6. Similar to Huebschmann's illustrations. The mass stained blue with crystal violet, dirty yellow with van Gieson's stain, and blue with Mallory's phosphotungstic acid stain. Hematoxylin and eosin. $\times 45$.

positive reaction. (2) Variations in the technic of staining with the crystal violet stain resulted in the presence of a pink color in the normal tissue control in four instances, whereas the simplicity of technic in staining with the methyl green was such that on no occasion was the normal tissue off color. (3) Despite a conscious effort to decolorize adequately the sections stained with Congo red, the control normal tissue retained some of the pink dye in 14 of the 24 sections. This color was usually located in the sclerotic arteries of the atrophic ovary. Because of this tendency for nonamyloid-bearing tissue to retain the dye, the Congo red stain was not considered reliable in the differential diagnosis of

amyloid. (4) The tendency of the iodine-stained section to fade made it desirable to examine the sections immediately after they were stained and again two and four days later, after fading had occurred. A grade 4 reaction in the recently stained section was characteristic, but did occasionally fade rapidly and four days after staining, it might be interpreted as doubtful. The khaki or mahogany color of the faded grade 2 reaction was in some instances more

TABLE 1
HISTOLOGIC CHARACTERISTICS AND TINCTORIAL REACTIONS IN 15 CASES OF AMYLOID TUMOR

CASE	DATE	FORM IN SECTION STAINED BY H & E	CELLULAR INFILTRATION	CV*	MG	CR	I
1	1916	Mainly flakes, some masses, glands	Slight; mainly plasma cells, occasional giant cells	2	2	2	2
2	1918	Flakes	Slight; small round cells	3	2	1	0
3	1918	Flakes	Slight; plasma cells	3	3	2	2
4	1926	Flakes	Insignificant	1	2	1	0
5	1929	Flakes	Insignificant	3	3	2	2
6	1931	Flakes and masses, inflammatory exudate	Polymorphonuclear cells	4	1	2	4
7	1936	Flakes and masses	Many giant cells, few plasma cells	4	4	4	4
8	1940	Fragment of tissue only	Insignificant	1	1	3	0
9	1941	Flakes	Insignificant	1	1	2	3
10	1941	Flakes and masses, calcium, osteoid tissues	Moderate; plasma cells and giant cells	4	3	2	3
11	1941	Flakes and masses	Few giant cells, few lymphocytes and plasma cells	3	3	2	3
12	1942	Flakes	Slight; giant cells	1	1	1	4
13	1913	Flakes	Insignificant	2	1	3	4
14	1943	Flakes	Insignificant	3	1	1	3
15	1916	Flakes and masses	Many giant cells, many polymorphonuclear cells	4	4	4	4

* CV indicates crystal violet; MG, methyl green; CR, Congo red; I, iodine. Tinctorial reactions were graded on a basis of 1 to 4.

characteristic and easier to interpret than was the dirt-brown color of the more recently stained tissue.

It was evident from the information contained in Table 1 that for a given tissue the intensity of staining with the different stains was roughly the same. The crystal violet stain was considered to be the most satisfactory stain. However, in Case 9, the reaction with the crystal violet stain was grade 1 and might conceivably have been considered negative had not the iodine reaction been grade 3. In Case 8 the amount of amyloid present in the fragment of tissue available for study was minimal and would certainly have been passed over in the crystal

violet-stained section had not its location been made evident by the intense reaction in the section stained with Congo red.

By observation of the same structure in the slides stained with different stains (the advantage of having serial sections), it was noted that if an area stained intensely with one stain, that same area frequently was intensely colored with the other stains. Thus, a given ring in a round mass of amyloid might stain intensely with crystal violet, methyl green and iodine. The Congo red tended to stain the amyloid with less local variation.

Contrary to expectation, essentially the entire mass of tissue which showed the characteristic appearance of amyloid in the section stained with hematoxylin and eosin, reacted as amyloid with the so-called amyloid stains. More particularly was this true of the sections stained with crystal violet. In no instance was there primarily vascular involvement with the bulk of the tumor mass staining as a nondescript material. In no instance was a very significant part of the tumor composed of a material which did not stain as amyloid.

It was evident from the information contained in Table 1 that the intensity of the staining reaction of amyloid was not dependent on length of preservation of the tissue. However, it was noted that when a frozen-section of fresh tissue from an amyloid tumor of the pharynx not included in this series was stained with crystal violet, the entire homogeneous mass stained a striking bright ruby red. The tissue was then fixed in 10 per cent formalin and embedded in paraffin. When sections were stained with crystal violet seven days later, only a grade 1 color reaction was present. Therefore, although fixation of an amyloid-bearing tissue in formalin may appreciably alter the intensity of the staining reaction of the amyloid, there is probably no progressive decrease in, or change in, the staining reaction over a period of years.

Results. From these observations, two constantly associated characteristic findings were considered the two criteria which must be fulfilled before the diagnosis of amyloid tumor may be made by the microscopic examination of tissue: (1) The "tumor" must be made up mainly of "flakes" or "concentric layered masses" or both, and (2) these flakes or masses must stain characteristically with one or more of the so-called amyloid stains.

The diagnosis of amyloid degeneration was confirmed by this study in 15 of the 28 cases reviewed.

In 7 cases excluded from this group, the tissues examined did not present the described characteristic histologic appearance of the amyloid tumor and did not stain as amyloid with the so-called amyloid stains. In an effort to differentiate these lesions more completely from the amyloid group, sections were stained with Mallory's phosphotungstic acid stain. The hyaline material present in these 7 tissues stained blue with this stain, whereas amyloid stained a rather nondescript brownish pink. It was considered that the homogeneous areas were in reality hyalinized fibrin or tissue exudate. Clinically, these lesions were noted to be benign pedunculated or polypoid nodules with the prognosis of any similar appearing lesion.

Huebschmann⁶ described fibrinohyaline deposits in laryngeal nodules, which

he considered to be probably the early stage of amyloid formation. Clinically, these lesions varied from the size of a pinhead to the size of a pea. The diagnosis was in most cases polyp, papilloma or fibroma. As well as he was able to determine, the symptom, hoarseness, disappeared soon after operative removal of the lesion and did not recur. Microscopically, these nodules were characterized by an intact epithelial surface stretched over edematous connective tissue containing dilated capillaries. Homogeneous masses of greater or lesser extent were present frequently in close association with a blood vessel. The masses stained brown or yellow with van Gieson's stain. The staining reaction with methyl violet he described as being negative with the smaller structures, but here and there in the larger masses there was present a weak reddish color. A stronger methyl violet reaction was present in one case only. Huebschmann expressed the belief that there was demonstrated a gradation from an entirely negative methyl violet-staining reaction to a positive reaction in lesions which appeared similar in structure.

The structure in the 7 cases in this series in which the lesions were considered to be hyalinized fibrin or tissue exudate appeared identical to the structure of the lesions that Huebschmann described (Fig. 6). The homogeneous masses stained dirty yellow with van Gieson's stain. They did not present any reddish areas when stained with the crystal violet stain. Mallory's phosphotungstic acid stain similarly differentiated these masses from true amyloid. Whether these lesions represented an early stage of formation of amyloid, as suggested by Huebschmann, could not be determined. The important observation was that, clinically, the lesions were easily removed and did not recur; and so, prognostically they differed from those lesions considered to be definitely amyloid.

One case excluded from the group, presented the microscopic appearance of a granulomatous reaction about a hyaline mass. This mass did not stain as amyloid. The lesion, clinically, was a contact ulcer granuloma of the vocal angle.

One case excluded from the group, presented the characteristic appearance and staining reaction of hyalinized collagen (it stained red with van Gieson's stain) and was considered probably an inflammatory tumefaction of the peribronchial lymph nodes with involvement of the bronchi.

Four cases were excluded because of inadequate material for proper study.

COMMENT

Microscopic findings. The histologic character of the amyloid tumor, as observed in this study, was uniform for all cases. The amyloid was found to be a relatively homogeneous eosinophilic material present in the tumor in the form of "flakes" or "concentric layered masses". Amyloid material has been universally considered to be a relatively homogeneous "hyaline" material. Many authors have emphasized the primary involvement of the vessel walls. Involvement of the vessel walls in this series, although certainly present, was not at all striking. The German authors particularly have described the involvement of the basement membranes of the mucous glands. Glandular involvement was impressive

in this series. The masses or clumps of amyloid material, described by many authors and believed by some to have been derived from vessels infiltrated by amyloid material, would appear in part at least to be derived from the infiltration of the mucous glands. Many authors have considered the presence of giant cells to be characteristic of the amyloid lesion. In this study giant cells were observed in 6 of the 15 cases. The more common cellular reaction was a small round cell infiltration, frequently with many plasma cells present. Whether this cellular reaction was a response to the irritation of the amyloid particles, or whether the amyloid was formed or deposited in the region of a pre-existing cellular infiltration was not apparent. A finding which was not emphasized in the descriptions reviewed, but which was considered to be characteristic of this series, was the fragmentation or flaking of the homogeneous amyloid material. It has been of interest that this fragmentation, so characteristic of the group of amyloid tumors studied, was noted only once in a review of a series of 31 cases of secondary amyloidosis, 7 cases of primary amyloidosis and 3 cases of amyloidosis associated with multiple myeloma. In one of the latter cases an amyloid nodule in the wall of the esophagus presented the characteristic flaked appearance.

Special staining reactions. Many authors have observed that amyloid, not "secondary" in nature, has tended to react atypically to the so-called amyloid stains. Some authors have made a diagnosis of amyloid tumor although the "amyloid" material reacted as normal tissue to these stains.

During the course of this investigation, the sections of tissue stained with the special stains were studied before the histologic character was noted in the sections stained with hematoxylin and eosin. Fifteen of the tissues reacted to the special stains differently than did the remainder of the tissues or the normal tissue control. The color reactions were not those that one would expect secondary amyloid to display. That is, they were atypical amyloid color reactions.

When the histology of the tissues was studied, it was noted that each of the 15 tissues which had given an atypical color reaction with the special stains presented the histologic features which have just been described as characteristic of amyloid in the tumors. Further, it was noted that no tissue which reacted as normal tissue with the special stains, presented the histologic features described as characteristic of amyloid. It was, therefore, considered that both the histologic features described and the atypical amyloid-like staining reactions were characteristic of this form of amyloid.

Many of the amyloid tumors described have been yellow. Virchow¹⁶ observed that amyloid was a translucent grayish or colorless material. He considered that the translucent nature of the material permitted the color of the neighboring structures to glimmer through and that the apparent color of the amyloid deposit was not a part of the amyloid itself. Those sections of tissue stained with Sudan III in this study demonstrated the majority of the stain to be in the pattern of the amyloid material, and relatively small amounts to be in the intervening connective tissue. It was considered that the material which absorbed the Sudan III and which was itself soluble in fat solvents was the site of the yellow pigment; that is, that the yellow color was present in association with the amyloid itself

and not in fatty deposits in the connective tissue in which the amyloid had been deposited.

SUMMARY AND CONCLUSIONS

Data on 28 cases with the diagnosis of amyloid degeneration of the larynx, trachea or bronchi were found in the surgical files of the Mayo Clinic. Adequate material for a satisfactory pathologic study was available in 24 cases. The diagnosis of amyloid "tumor" was confirmed in 15 cases.

The pathologic features considered characteristic of the tumors were as follows: (1) The homogeneous amyloid material occurred mainly in the form of "flakes" or "concentric layered masses", and (2) this amyloid material reacted characteristically with one or more of the so-called amyloid stains.

Of the special stains used, the crystal violet stain was found to be the most satisfactory for the identification of amyloid material.

Although fixation of amyloid-bearing tissue in formalin has been shown to alter the intensity of the stain reactions of the amyloid appreciably, there was no evidence of a progressive decrease in, or change in, the staining reactions over a period of years.

In seven cases the deposits appeared identical with those described by Huebschmann who called them "fibrinohyaline" deposits in the larynx.

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THE CONCEPT OF HEPATIC CLEARANCE*

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It is generally conceded that the available technics for measuring liver function leave much to be desired. These tests are only roughly specific for the various disease groups, and the results cannot be used for quantitative comparisons. In order to duplicate the relative precision attained with renal clearance studies⁷ and in order to attempt an elucidation of hepatic function comparable to that which has been accomplished with the kidney, a comparable mathematical expression for hepatic "clearance" has been derived. While studies on hepatic clearance may not yield data precisely of the same nature as those obtained on renal clearance, the data obtained by this type of study should make it possible to evaluate quantitatively, *in vivo*, the various distinct liver functions.

In the measurement of renal clearance, the problem is somewhat simplified by the fact that the material cleared appears in the urine where it can then be easily measured. Obviously, bile is not as easily or accurately obtained as is urine, and, furthermore, were we to limit ourselves to a study of biliary excretions, we would be neglecting the study of those substances cleared by the liver which do not appear in the bile. Therefore, the following derivation is based on the changes occurring in the plasma concentrations of any intravenously administered compound whose removal is accomplished by the liver.

DERIVATION

Let P = an initial plasma concentration,

P' = a final concentration greater than zero,

Pm = the mean concentration during the time interval between P and P' ,

C = the volume of plasma cleared during this time interval,

V = total volume of the fluid compartment containing the dissolved material,

Q = initial quantity present in solution,

Q' = final quantity present in solution.

The quantity of material removed during the clearance period will equal

$$C \times Pm$$

Then,

$$(1) \quad Q - CPm = Q'$$

Dividing both sides of the equation by V :

$$(2) \quad Q/V - CPm/V = Q'/V$$

* Received for publication, May 22, 1948.

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Since $Q/V = P$, and $Q'/V = P'$:

$$(3) \quad P - CP_m/V = P'$$

By rearranging equation (3):

$$(4) \quad (P - P')/P_m = C/V$$

Since the concentration of intravenously injected material should fall along a logarithmic curve, P_m is approximately equal to the geometric mean of P and P' .

$$(5) \quad P_m = \sqrt{P \times P'}$$

Substituting (5) in equation (4):

$$(6) \quad (P - P')/\sqrt{PP'} = C/V$$

Since determinations of plasma volume are unsatisfactory, and in order to avoid surface area calculations in comparing data, the quantity C/V has been kept intact. This algebraic expression symbolizes the fraction of the fluid volume which is cleared during the clearance period. Throughout the remainder of this paper, the expression "hepatic clearance" will represent the *fraction* of the total fluid volume (or solvent compartment) cleared in a sixty minute interval. The quantity C/V is then a *fractional clearance*.

APPLICATION OF THE CLEARANCE FORMULA

It can be seen from the derivation that the minimum data required for the calculation of fractional clearances are two plasma levels of an injected compound taken at the beginning and the end of a known time interval. The initial level is then substituted in the formula for P , and the final level is substituted for P' . The resulting fractional clearance, C/V , is then multiplied by an appropriate factor to obtain the fractional clearance for sixty minutes.

The actual operation of this method may be demonstrated by the following example:

BSP (bromsulphalein) level 5 minutes after injection = 60 per cent

BSP level 15 minutes after injection = 10 per cent

$(P - P')/\sqrt{PP'} = (60 - 10)/\sqrt{600} = 2.0 = C/V$ for 10 minutes.

$2.0 \times 6 = 12.0 = C/V$ for 60 minutes

Obviously, more than two determinations should be made of the time-concentration relationship in order to minimize the effects of experimental error.

POTENTIAL SOURCES OF ERROR

In the utilization of the fractional clearance formula, there are three potential sources of error: (1) the assumption of relative constancy in fluid volume under different conditions, (2) the assumption that the removal of the injected compound is accomplished solely by the liver, and (3) the assumption that the time-concentration relationship always follows a simple logarithmic curve.

It is probable that during the course of some diseases the actual function of the

liver as represented by C might well remain constant, but if there were inter-current edema or ascites, the fluid compartment represented as V would increase. If serial determinations of C/V were made while the edema was increasing, there would be a corresponding decrease in this calculated value for compounds present both in plasma and extravascular fluid.

The second and third assumptions are closely related and may be responsible for possible errors in the fractional clearance values presented in this paper. Some compounds may be cleared from the plasma by the reticulo-endothelial system and also to some extent by the kidney. However, significant errors of this type are more likely to occur with compounds diffusing out of the blood stream more slowly than they are excreted, but at a rate sufficiently large to produce a deviation from a true logarithmic curve. The calculated fractional clearance would then be distorted by the inclusion of the rate of diffusion out of the blood stream, and also to a lesser extent by the deviation of the calculated geometric mean from the true mean of the plasma concentration. In the ap-

TABLE 1
MEAN FRACTIONAL CLEARANCE IN THE ABSENCE OF LIVER DISEASE

COMPOUND	MEAN FRACTIONAL CLEARANCE	NO. OF CASES	STANDARD DEVIATION	COEFFICIENT OF VARIABILITY	REFERENCE NUMBER
				<i>per cent</i>	
Sodium d-lactate.....	2.99	8	0.71	26	(8)
Galactose.....	3.6	6	0.94	26	(2)
Galactose in dogs.....	3.7	10	1.34	36	(3)
Bromsulphalein (BSP)...	11.0	25	4.8	44	(5)
BSP in dogs.....	12.8	10	2.92	23	(3)
Bilirubin.....	1.3	6	0.35	27	(4)

plication of this method, these last two sources of error may be minimized or completely eliminated by determining the plasma concentrations at some time interval after equilibrium has been established between the plasma and the tissues.

It must also be clearly understood that the preceding derivation involves an approximation in the use of the geometric mean. While this will generally be more accurate than the arithmetic mean, it still does not completely coincide with the true mean which would equal the integral of P divided by the number of time units to which P is integrated. Furthermore, fractional clearance is more precisely defined as indicated in the following equation:

$$C/V = K (\log P - \log P') / (t \log e)$$

The error involved in using the simpler formula has been checked and found to be negligible in most cases. The simplified derivation is used here because of the ease in presentation and because of its ready correlation with clinical concepts.

It might well be objected at this point that these measurements of hepatic clearance are in no way different from those measurements already in use. In

one sense, this is quite true. Actually, the compounds used and the techniques for their analysis are unchanged, and the logarithmic equation above is quite similar to the equation for the "galactose removal constant" as derived by Colcher, Patek, and Kendall.² The important point is that the interpretation of the results is different. Instead of dealing with abstract, empirical constants, we now have a concrete expression related to actual mechanisms. Furthermore, results obtained with different tests are expressed in terms more suitable for comparison with each other, and the criteria for evaluating the usefulness of any given test or series of tests may be more precisely defined. At present, the quantitative evaluation or comparison of the effects of diseases and toxic or therapeutic agents on the liver is not really possible. The various tolerance and retention tests which are expressed quantitatively can be used for comparisons in a crude way, but, as will be demonstrated below, these comparisons are inherently grossly inaccurate and virtually useless.

TABLE 2
COMPARISON OF NORMAL AND ABNORMAL FRACTIONAL CLEARANCES

CONDITION	BROMSULPHALEIN	GALACTOSE	LACTATE	BILIRUBIN	REFERENCE NUMBERS
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
Normal	100	100	100	100	
Acute hepatitis	21	33	31	—	(2, 2, 8)
Cirrhosis	26	44	—	32	(2, 2, 4)
Chronic passive congestion	42	45	—	—	(2, 2)
Arsenical hepatitis	100	—	36	39	(4, 8, 4)
CCl ₄ in dogs (2 doses)	46	105	—	—	(3, 3)

COMPARISON OF HEPATIC CLEARANCES

In order to test the utility of this equation, various data have been abstracted from several papers in the literature dealing with studies on liver function. In some of these, the precise accuracy of the data and the number of cases or determinations fall somewhat short of ideal, but they have been taken as a reasonable first approximation of the constants required for comparisons. In Table 1, the normal fractional clearance values for four compounds are presented along with the statistical data essential for evaluating their validity. In the last column, the reference numbers indicate the sources of the data used in calculating the fractional clearances.

In Table 2, the fractional clearances present in several types of liver disease are presented. These are presented as per cent of normal in order to facilitate comparisons. The reliability of the data in Table 2 is impaired by the variety of sources involved; therefore, in Table 3 bromsulphalein (abbreviated as BSP) and galactose clearances in several conditions are summarized from tests performed on the same persons. In order to demonstrate the effect of diseases on dissociation of these functions, the fractional clearances of BSP are divided by

the fractional clearances of galactose in each patient. The means of these individual ratios are presented in the third column. The mean fractional clearances for each group are presented in the sixth and seventh columns.

DISCUSSION AND CONCLUSIONS

From the data in Table 1, it can be seen that of the four compounds studied, BSP is the most rapidly removed. In the period immediately following injection, this is partly attributable to the removal of BSP by the reticulo-endothelial system as demonstrated by the studies of Mills and Dragstedt.⁶ This objection to the fractional clearance values obtained with BSP may be eliminated by post-saturation studies as indicated in the previous discussion on potential sources of error.

The remainder of the data in Table 1 is also subject to revision as technics

TABLE 3

RATIOS OF BROMSULFALEIN: GALACTOSE CLEARANCE DEMONSTRATING DISSOCIATION OF BROMSULFALEIN AND GALACTOSE CLEARING FUNCTIONS IN DISEASE

CONDITION	NO. OF CASES	MEAN RATIO BSP:GALACTOSE	STANDARD DEVIATION	COEFFICIENT OF VARIABILITY	MEAN BSP	MEAN GALACTOSE
				<i>per cent</i>		
Normal.....	6	3.5	0.86	25	11.75	3.47
Chronic passive congestion.....	9	2.9	0.81	28	4.61	1.59
Acute hepatitis.....	5	1.8	0.60	33	2.32	1.32
Compensated cirrhosis..	7	1.8	0.29	16	2.86	1.60
Decompensated cirrhosis.....	18	3.0	1.30	42	3.34	1.23

and procedures are improved, but all of these data are of value as standards for comparing tolerance and retention data already obtained with clinical material that is difficult or impossible to duplicate.

The data in Table 2 are presented chiefly as an illustration of the potential usefulness of fractional clearance studies in the investigation of liver diseases. The figures given represent an over-all picture of groups with the same type of disease, but there is no correlation here with the stage of diseases, and it is certainly reasonable to believe that wide variations will occur between different stages of the same disease and perhaps with different modes of therapy.

Similar objections might be made to the data of Table 3, but here the variability of the BSP: galactose ratio is offered as a substantial proof of the major postulate in the concept of hepatic clearance. Drill and Ivy³ have concluded from their studies that there is an association rather than a dissociation of hepatic functions such that any given etiologic agent producing liver disease will eventually distort all of the functions. The utility of the concept of hepatic clearance as presented here is dependent on the postulation of a dissociation of these functions so that different functions tend to be distorted to different degrees

by different diseases. Careful consideration of these ideas will make it quite clear that fundamentally the concept of association might be correct; obviously, ablation of any given portion of the hepatic cells must be followed by a corresponding diminution in maximum capacity of all of the functions. In any given case, it would not matter whether the ablation were produced by a cautery, a virus, contracting fibrous tissue, or carbon tetrachloride. The apparent obvious truth of this idea still does not exclude the possibility of dissociation of functions in a damaged cell which has not yet become necrotic and which may even recover. Examination of the data of Drill and Ivy reveals their data to be equally consistent with this idea, and the data in Table 3 offer further quantitative substantiation. If the functions were completely associated, the BSP: galactose ratios would be constant, but in Table 3 they are not. It is clear then that: (1) Dissociation here implies a *diminished degree* of association of functions, (2) the degree of dissociation may vary with different diseases, but (3) the degree of dissociation will probably depend on the stage or the severity of the disease as well as the type. The studies of Soskin⁹ on glucose metabolism and the endocrine investigations of the Biskinds¹ have led us to the realization that there are subtler degrees of liver dysfunction than are manifested by our present methods of measuring liver function. It is, therefore, reasonable to suspect that there also may be subtler varieties of liver disease than we have been able to recognize by our present methods of investigation. With further development and technical refinement of hepatic clearance methods, it may be possible to evaluate these slight degrees of liver dysfunction both qualitatively and quantitatively.

Aside from these almost purely investigative aspects of the clearance concept, the formula presented has certain immediate practical value in clinical studies. Tolerance and retention data as now reported do not allow comparisons between two tests even when performed on the same individual at different times under different states of health. Thus, for example, a BSP retention of 20 per cent does not indicate that there is twice as much liver damage as there is when the retention is 10 per cent. This is further demonstrated by an example in which three galactose tolerance tests were performed on the same person at different times during an episode of acute hepatitis. The galactose tolerance data are summarized as follows:

(1) second day of jaundice.....	164	139	104	69	53	28
(2) ten days later.....	188	145	104	57	56	—
(3) three months after recovery.	131	69	20	0	0	—

These levels are taken at 15 minute intervals, the first being taken 15 minutes after injection, and the last 90 minutes after injection. The improvement in these tolerances appears obvious by inspection, but the evaluation is qualitative and not really adequate for comparison of the stages with each other or with other cases. On the other hand, the fractional clearances calculated from the first three levels (using a midpoint value in place of a calculated geometric mean) are as follows:

(1) second day of jaundice.....	1.2
(2) ten days later.....	1.2
(3) three months after recovery.....	3.2

In each figure there is a convenient and accurate summary of the situation, and it is also possible to state that on the second and 12th days of this patient's jaundice, the galactose clearing activity was 33 per cent of normal. This illustration demonstrates the clinical utility of this concept as well as its value as an investigative tool.

SUMMARY

1. A simplified formula for the calculation of fractional hepatic clearance has been derived. With this formula, the fraction of the fluid volume cleared by the liver of an injected compound may be determined.

2. Precise quantitative comparisons of liver functions with tolerance or retention data as now reported cannot be made unless the data are recalculated as fractional clearances.

3. The concept of hepatic clearance as presented here is based on an assumption of dissociation of hepatic functions such that different diseases distort different hepatic functions to different degrees.

4. This concept and the underlying assumptions are substantiated by the fractional clearance values which have thus far been determined.

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APPENDICEAL LESIONS IN THE PRODROMAL STAGE OF MEASLES*

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The occurrence of lesions in measles, other than in the skin, was first demonstrated by Ciaccio³ in 1910 and Alagna¹ in 1911. Ciaccio³ saw large multinucleated giant cells resembling megakaryocytes in the lung, while Alagna,¹ who confined his studies to the mucous membranes of the nose and throat, noted large multinucleated giant cells in the tonsils. In 1931 Warthin¹⁸ and Finkeldey⁶ independently described the presence of multinucleated giant cells in the tonsils in the prodromal stage of measles. They described these giant cells in considerable detail and, by common usage, these multinucleated cells have since been called Warthin-Finkeldey giant cells. In 1932 Finkeldey⁷ reported the presence of similar giant cells in the so-called germinal centers of the lymphoid tissue of the appendix in the prodromal stage of measles. His case concerned an 8 year old boy in whom the measles rash appeared two days after appendectomy. In the same year Herzberg¹¹ reported similar findings in a 6 year old child in whom the typical measles rash developed four days after appendectomy. Since 1932, isolated and small series of cases have been reported by Davidsohn and Mora,⁵ Fischer,⁸ Schultze,¹⁵ Bullowa *et al.*,² Wegelin,¹⁹ Newman and Milstead,¹³ Mulligan¹² and Corbett.⁴ In all of these cases the measles rash appeared on the day of operation or up to four days after operation or on the day of death. (See Table 1.)

The total number of reported cases of this characteristic lesion in the appendix is rather small, and it is believed that many experienced pathologists in general hospitals having a small pediatrics service have never encountered this lesion in their material. Furthermore, because of the abdominal pains which, not infrequently, are present in the prodromal stage of measles and, more important, because acute appendicitis may be present either before or on the day the rash appears, it seems desirable to call attention again to this condition.

We have encountered the lesion in four patients, in each of whom appendectomy was performed before the typical measles rash appeared.

REPORT OF CASES

Case 1

Clinical data. A 7 year old boy was admitted to the hospital on February 20, 1942, with the complaint of paraumbilical pain and vomiting two days prior to admission. The rectal temperature was 100.2 F., there was slight but generalized lymph node enlargement and slight tenderness in the right lower quadrant. No leukocyte count was recorded on the chart. A pre-operative diagnosis of subsiding appendicitis was made, and he was operated on the following day. At operation the serosal vessels of the appendix were injected. In the mesentery of the small bowel several enlarged lymph nodes were seen. The postopera-

* Received for publication, May 3, 1948.

tive course was uneventful, and on his third postoperative day a typical measles rash appeared over the face and body.

Pathologic report. The serosal surface of the appendix was gray, smooth and glistening, and the subserosal vessels were prominent. Each of three transverse sections of appendix taken from different levels showed an intact mucous membrane. The lymphoid tissue was hyperplastic, and in many secondary nodules or so-called germinal centers multi-nucleated giant cells were encountered. The giant cells contained from 3 to 20 nuclei and appeared to be composed of fused lymphocytes or endothelial cells. The nuclei were uniform in appearance, and the chromatin arrangement appeared to be similar to that of adjacent lymphocytes. The cytoplasm was scanty, and neither nuclear nor cytoplasmic in-

TABLE 1

OCURRENCE OF GIANT CELLS IN THE APPENDIX IN 20 PATIENTS WITH MEASLES IN RELATION TO THE NUMBER OF THE POSTOPERATIVE DAY OF APPEARANCE OF RASH

AUTHOR	NO. OF PATIENTS	NUMBER OF POSTOPERATIVE DAYS OF APPEARANCE OF RASH
Finkeldey (1932)	1	3
Herzberg (1932)	1	2
Davidsohn and Mora (1932)	1	4
	1	3
	1	2 days before operation
Fischer (1933)	1	1½
Schultze (1933)	1	2
Bullock et al. (1937)	3	All postoperative
Wegelin (1937)	1	4
	1	3
	1	Day of operation
Newman and Milstead (1940)	1	3
Mulligan (1944)	1	3
Corbett (1945)	1	Death on day rash appeared
Simon and Ballou (1948)	1	3
	1	4
	1	5
	1	5

clusion bodies were present. The giant cells were found not only in the secondary nodules (Fig. 2), but were also seen in the lymphoid tissue relatively close to the mucous membrane. The serosa was slightly edematous, and a few scattered lymphocytes and rare polymorphonuclear leukocytes were seen in the serosa in extravascular position.

Diagnosis. Subacute periappendicitis, minimal, in an appendix, the seat of an acute infectious exanthem, probably measles.

Case 2

Clinical data. An 8 year old girl was admitted to the hospital on April 13, 1943, for investigation. Ten days later, on April 23, she complained of abdominal pain and was found to have right lower quadrant pain and rebound tenderness. Her rectal temperature at this time was 101.4 F., and the leukocyte count had risen from 8800 to 11,000 per cu. mm.

At operation, which was carried out the same day, considerable free fluid was found in the peritoneal cavity, which proved to be sterile upon culture. Mesenteric adenitis, most prominent about the ileocecal junction, was noted, and the lymph nodes were large and suc-

culent. The appendix did not exhibit any gross acute inflammatory changes. There were some adhesions about its base, and the tip was bulbous.

On the third postoperative day the child developed Koplik's spots. She was discharged from the hospital and on the next day developed a typical measles rash. She made an uneventful recovery.

Pathologic report. The appendix presented a gray, smooth and glistening serosal surface, the lumen was empty and the wall was not remarkable. Three transverse sections, taken from different levels, showed numerous pinworms within the lumen. The mucous membrane was intact. The lymphoid tissue was hyperplastic, and within practically every secondary nodule anywhere from 1 to 20 giant cells were encountered (Fig. 1). The giant cells each contained from 3 to 80 nuclei (Fig. 3) which were slightly hyperchromatic and resembled the nuclei of adjacent lymphocytes in size, shape and chromatin arrangement. The cytoplasm of the giant cells was extremely scanty. These giant cells appeared to be formed by the fusion of adjacent lymphocytes (Fig. 4). While the majority of the giant cells was found in the secondary nodules of the lymphoid follicles, a few lay outside the follicles close to the mucous membrane. The submucosa was fibrous, and a few dilated blood vessels were seen in the serosa. In no situation were inflammatory cells seen.

Diagnosis. *Enterobius vermicularis* infestation of appendix.

Comment. From the appearance of the giant cells in the secondary nodules of the lymphoid tissue, it is apparent that this patient either has, has recently had, or is now about to have, measles or chickenpox.

Case 3

Clinical data. A 7 year old boy was admitted to the hospital on February 26, 1948, with abdominal pain. Physical examination revealed right lower quadrant tenderness with "défense musculaire". His leukocyte count was 5500 per cu. mm., and his rectal temperature was 101 F. At operation, that same day, free fluid was encountered in the abdomen. The appendix did not exhibit any acute inflammatory changes. The mesenteric lymph nodes were enlarged. On his second postoperative day he developed a cough, and his temperature rose to 102 F. On this day the leukocytes numbered 3750 per cu. mm. On the fifth postoperative day his temperature dropped to 99 F., his leukocytes mounted to 6600 per cu. mm. and he developed a typical measles rash.

Pathologic report. The serosal surface of the appendix was gray, smooth and glistening. Multiple sections showed a wall of average thickness and a patent lumen filled with dark brown fecal material.

Three transverse sections of appendix, taken from different levels, showed, in all, a lumen of variable size lined by intact mucous membrane. The lymphoid tissue was moderate in amount, and in many secondary nodules from 1 to 8 multinucleated giant cells were seen. These giant cells contained from 3 to 15 nuclei which resembled in size, shape and chromatin arrangement the nuclei of adjacent lymphocytes. Scarcely any cytoplasm was seen in these giant cells, and they appeared to be due to a fusion or agglomeration of naked lymphocytic nuclei. No giant cells were seen outside of the secondary nodules, and no inflammatory exudate was seen in any portion of the appendiceal wall.

Diagnosis. Appendix, in a case of acute exanthem. *Comment.* This child has or is about to have measles or chickenpox.

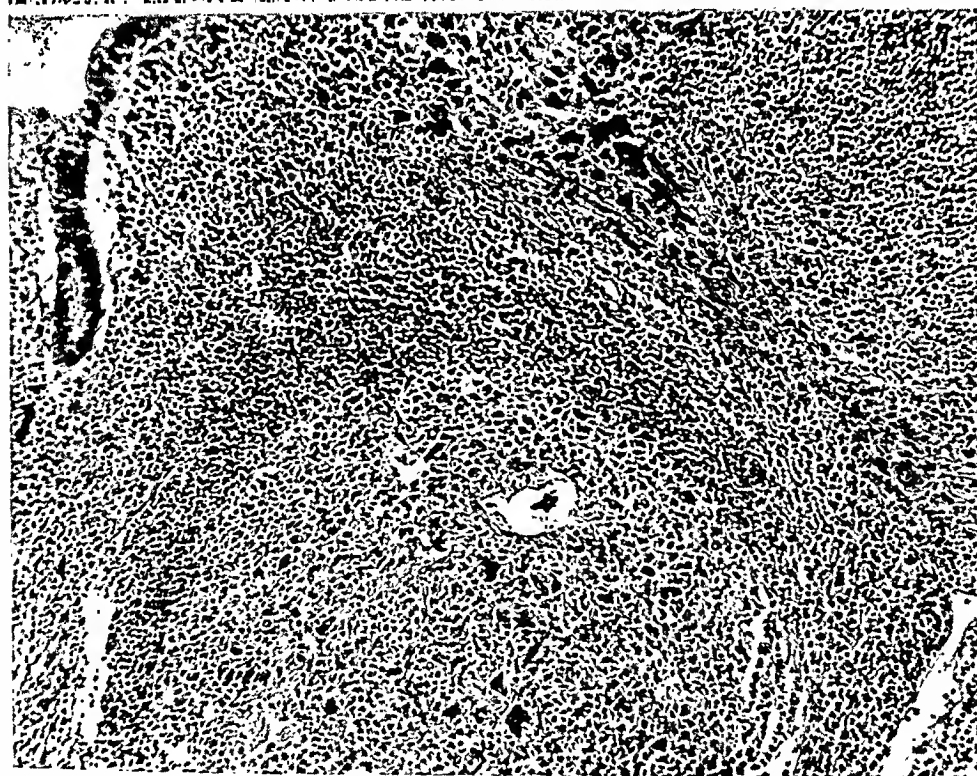
Case 4

Clinical data. A 10 year old boy was admitted to the hospital on March 21, 1948, with symptoms and signs typical of acute appendicitis. His leukocyte count was 13,000 per cu. mm., and his rectal temperature upon admission was 103 F.

At operation, carried out on the day of admission, the appendix was found to be acutely inflamed. On his first postoperative day his temperature rose to 104.3 F. and remained elevated for four days. By this time his leukocyte count had dropped to 6400 per cu. mm. On his fifth postoperative day he developed a typical measles rash.



1



2

FIG. 1. Case 2. Medium power view of segment of appendiceal wall. Note typical location and appearance of giant cells in secondary nodules of lymphoid tissue. $\times 49$.

FIG. 2. Case 1. This segment of appendiceal wall shows that giant cells may be found outside lymphoid follicle and relatively close to the mucous membrane. $\times 130$.

Pathologic report. The serosal surface of the appendix was slightly granular, and the subserosal vessels were moderately dilated. The wall was of average thickness, and the lumen contained a small amount of semi-solid brown fecal material. Three transverse sections of the appendix, taken at different levels, showed, in each, variable degrees of ulceration of the mucous membrane, hyperplasia of the lymphoid tissue, edema of the wall and a variable degree of infiltration of all the coats of the appendix by moderate numbers of lymphocytes and polymorphonuclear leukocytes.

Diagnosis: Acute appendicitis.

No typical multinucleated giant cells were seen in the original sections of this appendix. After the measles rash appeared, the balance of the appendix was blocked and sectioned. These sections showed moderate numbers of secondary nodules containing typical Warthin-Finkeldey giant cells.

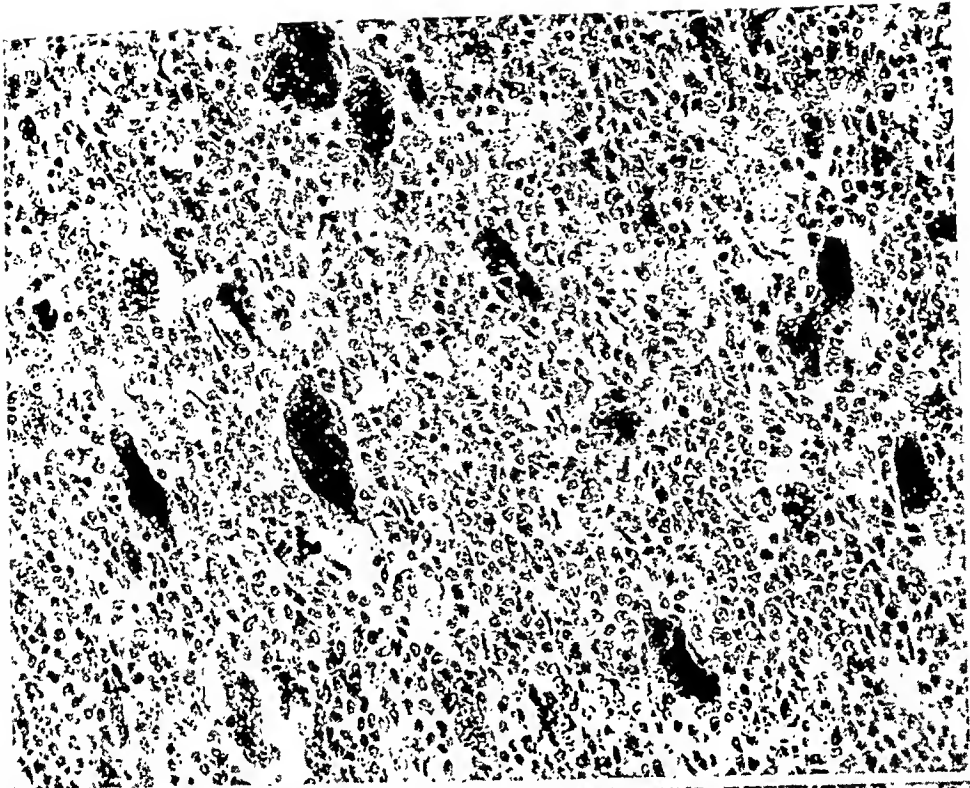
A fifth case was recently seen by us in which the appendix was removed nine days before the typical measles rash appeared. In this case, although careful search was made, no Warthin-Finkeldey giant cells were found.

DISCUSSION

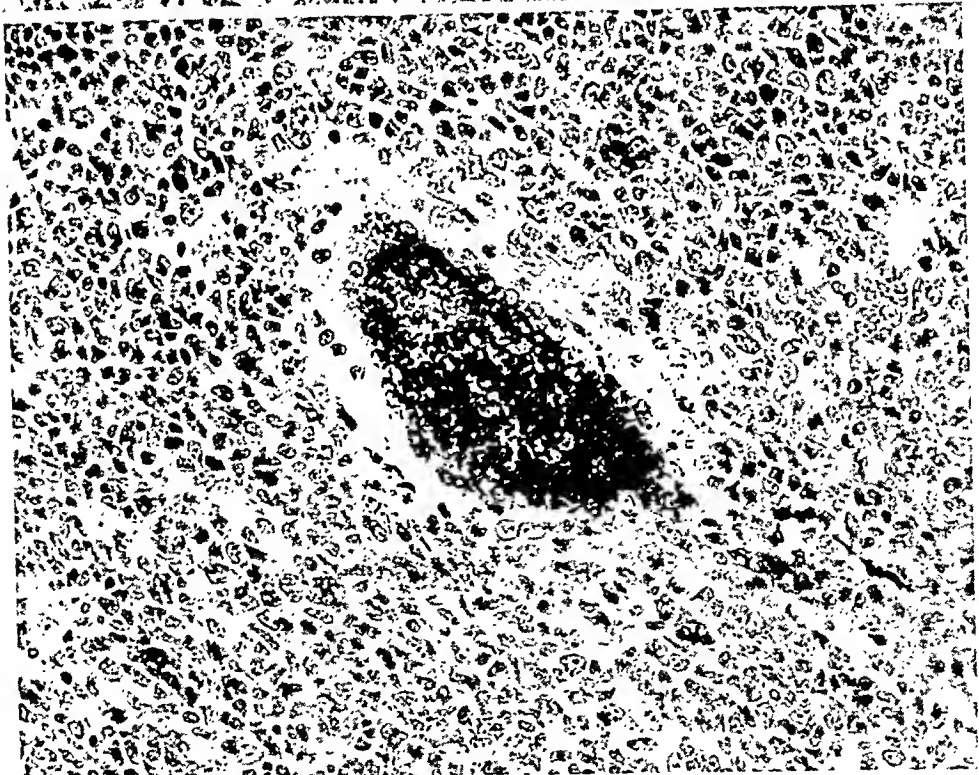
The multinucleated giant cells of the Warthin-Finkeldey type, described in the lymphoid tissue of the appendix of the foregoing cases, are similar in all respects to those previously and adequately described in the literature. These giant cells appear not only in the lymphoid tissue of the appendix, but in the lymphoid tissue throughout the body, and have been described in the lymphoid tissue of the entire alimentary canal, tonsils, spleen, intra-abdominal lymph nodes and thymus.

These multinucleated giant cells appear to be pathognomonic for the prodromal stage of measles. Indeed, Gordon and Knighton,⁹ on the basis of their studies on experimental measles, believe that "it is not unlikely that the Warthin-Finkeldey giant cell reaction is a much more sensitive indication of infection than are clinical manifestations". With the single exception of a case reported by Tomlinson,¹⁷ who described similar giant cells in the tonsils of a case of chickenpox, all reported cases appear to have occurred only in subsequently proved instances of measles. It is interesting to note that Gordon and Knighton⁹ and Corbett⁴ were inclined to doubt the diagnosis of chickenpox in the case reported by Tomlinson.¹⁷ It would appear, therefore, until additional evidence is available, that the presence of these multinucleated giant cells is specific for prodromal measles.

The time of initial appearance of these giant cells, in human beings, in relation to known exposure to measles is not definitely established. That they may appear relatively soon after exposure is suggested in the case reported by Hathaway.¹⁰ Hathaway's patient, age 2½ years, died of generalized fibrinopurulent peritonitis incident to acute appendicitis with perforation. Four days previously he had been exposed to measles. At autopsy multinucleated giant cells were found in the lymph nodes and spleen, but none were found in the appendix. In the experiments conducted by Gordon and Knighton,⁹ typical giant cells were found in the lymph nodes of monkeys as early as three days after inoculation of blood from measles patients. In this respect, we have observed a case in which



3



4

FIG. 3. Case 2. Center of a single secondary nodule showing dense nuclei making up giant cells. Detail is lost because of marked overlapping of nuclei. Giant cells here contain from 10 to 35 nuclei. $\times 260$.

FIG. 4. Case 2. Giant cell composed of over 80 countable nuclei. The nuclei of the giant cell are much darker, richer in chromatin than the endothelioid cells and approach the appearance of lymphocytes. $\times 340$.

an appendix was removed nine days before the measles rash appeared, and no giant cells were found in the lymphoid tissue of this organ. It appears certain, however, that by the fifth day before the appearance of the rash, the giant cells may be present in the lymphoid tissue of the appendix.

The duration of persistence of these giant cells in the appendix after the rash appears is unknown. In one of the cases reported by Davidsohn and Mora,⁵ giant cells were found in the appendix of a patient whose rash appeared two days before operation, but giant cells were absent in another patient in whom suppurative appendicitis occurred nine days after the measles rash had appeared.

Various opinions regarding the genesis of the Warthin-Finkeldey type of giant cell have been given by different authors. Warthin¹⁸ thought that they arose by amitotic division in hyperchromatic cells resembling lymphocytes. Finkeldey⁶ considered that these giant cells might represent a fusion of lymphocytes or might be derived from reticulum or even mesenchymal cells. Davidsohn and Mora⁵ suggested that the nuclei of these giant cells had features resembling plasma cells; and modifications of all these concepts have been reported by others.^{4, 5, 10-12, 16, 19} Mulligan¹² has offered the suggestion that "the multinucleated giant cells of the lymphatic tissue in measles are formed as the result of the polynuclear abnormal development of the stem cell paralleling the mononuclear normal development of the lymphocyte from the stem cell". From the study of our material, the theory of origin of these cells by fusion of lymphocytes seems to be the most plausible. The majority of the giant cells contain from 3 to about 30 nuclei, but Wegelin¹⁹ has described giant cells containing 100 nuclei. We have seen giant cells containing at least 80 countable nuclei (Fig. 4), but giant cells with very large numbers of nuclei appear to be the exception rather than the rule.

It is important to point out that the Warthin-Finkeldey type of giant cells found in the lymphoid tissue of the prodromal stage of measles is quite different from the fused epithelial giant cells found in the lungs and bronchi of various virus pneumonias of which measles is but one example, as was recently demonstrated by Pinkerton, Smiley and Anderson.¹⁴ These giant cells are quite different in appearance and, in addition, contain both cytoplasmic and nuclear inclusion bodies.

In all our cases appendectomy was performed during the pre-eruptive or prodromal period. There was no coryza or catarrh of the upper respiratory tract at the time the patients were examined. In two of our four patients, the appendix was free of inflammatory change and in three patients there was evidence of mesenteric adenitis. In two instances the operation was undertaken during an epidemic of measles. In fact, in Case 4 the patient had acute appendicitis and was accepted for admission because it was erroneously assumed that he had already had measles.

Bullowa *et al.*,² whose patients were from the Willard Parker Hospital, New York, reported 11 cases of appendicitis in a series of 6357 cases of measles. Five cases of appendicitis were noted in 2534 cases of chickenpox and only three in 6252 cases of scarlet fever. Of paramount importance in this series reported

by Bullowa *et al.*,² is the fact that in every one of the 11 patients with measles perforation of the appendix had occurred at the time appendectomy was performed. Two of these children died. In 2 of the 11 patients perforation had occurred in the prodromal period.

Acute appendicitis may occur at any time during the prodromal period or after the measles rash has appeared. On the other hand, pain and tenderness may suggest mesenteric adenitis, particularly as a manifestation of measles. The occurrence of an epidemic of measles or the possible presence of symptoms which may represent prodromal manifestations of measles should not be deterrents to operation when the clinical picture indicates acute appendicitis.

SUMMARY

Four cases have been presented in which appendectomy was performed in the prodromal stage of measles.

In three of the cases typical Warthin-Finkeldey giant cells were demonstrated in the appendix, which permitted a diagnosis of measles to be made before the rash appeared.

In two of the cases inflammatory lesions were present in the appendix at the time of operation.

In spite of the well-known symptoms of mesenteric adenitis which frequently accompany measles, acute appendicitis may occur at any time during the course of measles and warrants appendectomy.

Until proof is presented to the contrary, the presence of the Warthin-Finkeldey type of giant cell should be regarded as specific for measles. In all probability, this cell is produced by the fusion of lymphocytes.

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CORONARY SCLEROSIS IN INFANCY

REPORT OF THREE AUTOPSIED CASES, TWO IN SIBLINGS*

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Coronary arteriosclerosis is a rare condition in infants, but in recent years has been reported with increasing frequency. In 1946, Stryker⁵ collected from the literature 15 cases of coronary sclerosis in infants under one year of age and included a description of 5 others. In the same year Field² also listed the previously recorded cases and added one of his own. A later case, described by Hause and Antell,⁴ brings the total to 22.

Our report adds three cases. One case is of interest because more than the usual clinical history is available, and also because of the possibility of an allergic background. The other two cases are unique in that siblings are represented.

CASE 1

Clinical Data

A white male infant, weighing 9 pounds, 1 ounce, was born on September 12, 1946 at St. Margaret Memorial Hospital on the service of Dr. J. L. Kostyal. The mother, a 20 year old primipara, was in labor for 24 hours. A routine roentgenogram taken on the fifth day showed moderate enlargement of the mid-thoracic shadow and some enlargement of the heart. The baby was never breast fed. At the age of two weeks "Percomorph Oil", 5 drops daily, was started. When the baby was 36 days old he weighed 11 pounds, 1 ounce and the physical examination was negative. Regurgitation of feedings began at this time and continued for 11 days until tincture of belladonna, 1 drop, was given before meals. When the baby was 54 days old he seemed cranky and upset and following his 10 A.M. feeding began to make a grunting noise. The parents reported that the respirations increased gradually and that the infant's "color was bad". When seen by a physician that afternoon, November 5, 1946, the baby was cyanotic and appeared acutely distressed. The chest was clear, the heart rate was 130 per minute; the sounds were of good quality, and no murmurs were heard. The temperature was 99 F. The abdomen, slightly distended, became hard with each respiratory grunt. At 6:30 P.M. the infant appeared worse, cyanosis was increased and the grunting noises were more intense. The eyes were half opened. When the child was admitted to St. Margaret Memorial Hospital, an x-ray film of the chest revealed marked generalized enlargement of the heart shadow with clear lung fields. Despite oxygen therapy the baby died at 9:30 P.M. of that day.

Additional history indicated that the mother was well during pregnancy and had taken no vitamin D preparation. She did take dicalcium phosphate, 2 capsules daily, as well as an iron preparation. The infant's maternal grandfather died at age of 54 from "asthma and a heart attack".

Autopsy Findings

Gross description. Autopsy was performed ten hours after death. The infant measured 56 cm. in length and weighed 5440 Gm. (12 pounds). There were no significant external

* Received for publication, May 4, 1948.

findings. The *thyroid* and *parathyroids* were not examined. The *heart* was enlarged to the left, the apex occupying a position beneath the fifth rib, 1 cm. medial to the left anterior axillary line. There was a slight excess of clear amber pericardial fluid. A sample of heart's blood, on chemical analysis, gave the following results: serum cholesterol 125 mg. per 100 ml.; serum calcium 16.5 mg.; serum protein (falling drop method) 4.86 Gm.

The *thymus* weighed 39.5 Gm. and presented several petechial hemorrhages. The left *lung* weighed 59.5 Gm., the right 80 Gm. Both were firm and uniformly doughy to feel. Blocks from each lung sank in water. The cut surfaces were flat, bluish pink and moderately firm. The upper lobes were lighter and softer than the lower lobes. Vessels, bronchi and peribronchial lymph nodes were not grossly remarkable. The *heart* was enlarged, weighing 42 Gm. and measuring 7.3 x 5 x 3.5 cm. A minute subepicardial hemorrhage was present on the upper left ventricular wall at a point 1.7 cm. below the emergence of the anterior coronary artery. All of the superficial coronary branches were thickened, sclerotic and calcified. In an x-ray film made of the heart after removal, the main coronary branches were clearly delineated by virtue of calcific deposition within their walls. An irregularly oval, pale, yellowish pink zone of softening, measuring 1 cm. in surface diameter, was noted on the anterior wall of the left ventricle just above the apex. It was rimmed by a dark red border. The left ventricular wall measured up to 7 mm. in thickness, the right 4 mm. On section the lumina of the main coronary arteries appeared nearly obliterated. The valve orifices and leaflets were normal, except for a tiny petechial hemorrhage of the tricuspid curtain. There was thinning of the wall in the softened area, but there were no adherent clots. The foramen ovale was practically closed. The *aorta* appeared diffusely thickened. Both *internal iliac arteries* were thickened and sclerotic. Several *gastric and mesenteric arteries* were sclerotic. The lymph nodes of the small bowel mesentery were slightly enlarged. The liver, gallbladder, pancreas, spleen, kidneys, adrenals, urinary bladder, and testicles were of normal size and not grossly remarkable.

Microscopic findings. A few small lamellated basophilic calcific concretions were present in the thymus, distributed chiefly near Hassall's corpuscles. There was marked capillary congestion of the lungs. Many alveoli contained small amounts of edema fluid. Many red blood cells and monocytic phagocytes were scattered throughout the air sacs. A patchy intra-alveolar serocellular exudate was seen in which neutrophils were prominent. Several of the larger arterial branches showed basophilic calcareous thickening or coarsening of certain of their medial elastic fibers. In at least one instance this was accompanied by thickening of the internal elastic lamina. The musculature of the *heart* in the zone of infarction presented fragmentation, poor staining reaction and neutrophilic infiltration. Sections of each of the main *coronary* branches presented a similar type of diffuse thickening of their walls. This change characteristically involved all three coats. The lesion was typified by a peculiar fibroblastic intimal thickening with marked luminal narrowing. Just peripheral to the fibroblastic layer were plates and agglomerations of calcific material in an interrupted circumferential pattern. This calcareous material lay chiefly within the media (Fig. 1). Cellular infiltration, consisting of lymphocytes, plasma cells and eosinophils, was noted in the adventitial and periadventitial tissues. In a few sections eosinophils were present in large numbers. There were no thrombi although extreme luminal narrowing was illustrated. Intramyocardial coronary artery branches were not involved. In the *aorta*, there was calcareous thickening and degeneration of elastic fibers in the media. The fibers were involved singly or in groups. These fibers were chiefly in the outer portion of the media. An adventitial cellular infiltrate was noted in which eosinophils were conspicuous. The *mesenteric artery* presented changes almost identical with those in the coronary vessels. There was evidence of slight fatty metamorphosis of the *liver*. The *right renal artery* presented sclerosis similar to that in the coronary arteries.

About 2 per cent of the glomeruli of the *kidneys* were partially or completely hyalinized with thickening of Bowman's capsules. One or two structures resembling epithelial crescents were noted. An occasional glomerulus presented calcific degeneration and a rare collecting tubule showed calcific change. Several small focal collections of lymphocytes



1



2

FIG. 1. Case 1. Cross section of left coronary artery showing calcareous deposits in the media, and fibroblastic thickening of the intima. $\times 32$.

FIG. 2. Case 1. Cross section of a papillary muscle with infarction. The central area shows coagulation necrosis and the surrounding zone vaeuolation and beginning necrosis. $\times 270$.

could be seen in the interstitial tissue of the cortex. In one section of *adrenal* a small peripheral artery presented granular calcific thickening of its internal elastic lamina. Both *internal iliac arteries* were nearly obliterated by dense fibrous intimal thickening.

Less medial involvement was seen here than in previously described vessels. Sections of diaphragm, gastrointestinal tract, gallbladder, pancreas, spleen, prostate, testicles and sternal marrow were not remarkable.

Pathologic diagnoses: Generalized arteriosclerosis; medial sclerosis of coronary, pulmonary, internal iliac, gastric, mesenteric, renal and peri-adrenal arteries and aorta; coronary stenosis, all main branches; myocardial infarction, left ventricle; myocardial hypertrophy; pericardial effusion; chronic passive congestion of lungs; petechial hemorrhages of thymus and heart; bronchopneumonia; fatty metamorphosis of liver; calcific deposition in thymus and kidneys; mild advanced glomerulonephritis.

The other two patients were siblings with almost identical histories. A similar fatal outcome occurred in both at approximately the same age. These two infants were born and died in the same year; the first baby lived 53 days and the second child lived 70 days. A previous child had died under similar conditions at about the same age. The maternal lineage was Italian, the paternal Irish. The physician who attended the mother through her three pregnancies said she had exhibited no unusual signs or symptoms and that she had received no medication which might predispose or contribute to the vascular changes in the infants. Chemical analysis of the blood of the mother, taken after birth of the third child (Case 3) gave the following values: total protein, 8.39 Gm. per 100 ml.; albumin 4.46 Gm.; globulin 3.93 Gm.; phosphorus 2.9 mg. and calcium 10.6 mg. There was no familial history of asthma or other diseases pertaining to the condition found.

CASE 2

Clinical Data

T. A. P., the first child, a white male, was born January 1, 1946 and remained well until February 20, 1946 at which time he developed a low grade fever with rapid respirations. On February 23, the family physician gave him a sedative following which he appeared improved. However, because of abdominal distention and suspected intestinal obstruction, the physician sent him to the Children's Hospital of Pittsburgh. On arrival at the hospital, the child was markedly cyanotic and the respirations were slow and labored. Despite oxygen therapy, breathing ceased in a few minutes. The autopsy findings in this baby were almost identical with those observed in Case 3 and only the latter will be reported in detail.

Autopsy Findings

The autopsy was performed one hour after death. The body was that of a well developed and well nourished, 6 week old male measuring 55 cm. in length. There were no abnormalities noted on external examination. The head showed considerable molding and both fontanelles were widely open. The chief pathologic changes were in the heart and vessels. The heart was hypertrophied and weighed 40 Gm. All of the superficial coronary arteries were thickened and calcified. The left ventricular wall was slightly thicker than normal, measuring 6.5 mm. in thickness. The right ventricular wall measured 4 mm. in thickness. The valve orifices and leaflets were normal.

Pathologic diagnoses: Generalized arteriosclerosis; medial sclerosis of coronary, pulmonary, gastric, mesenteric, renal, hepatic and thymic arteries; atheroma of aorta; coronary stenosis; myocardial infarction, left ventricle; myocardial hypertrophy; enlarged thymus; parenchymatous degeneration of kidneys.

CORONARY SCLEROSIS IN INFANCY

CASE 3

Clinical Data

L. J. P., also a male, was born October 10, 1946. For a few days following birth this baby showed jaundice. He was well for about seven weeks and on the fifty-third day of life suddenly developed difficult breathing. The dyspnea persisted during the three-hour trip to the Children's Hospital where, on arrival, he was immediately put in an oxygen tent. He died in shock 30 minutes later. Since the baby was cyanotic and *in extremis* on admission, only a brief physical examination was made. Scattered rales were heard over the entire chest.

Autopsy Findings

Gross description. The autopsy was performed two hours after death. The body was that of a well developed and well nourished white male infant, measuring 50 cm. in length. The head showed considerable molding and both fontanelles were widely open. Otherwise the external examination was negative. The *thymus* was large, weighed 20 Gm., extended down to the auriculoventricular groove, and covered the upper part of the thoracic cavity. On section it appeared normal. The *thyroids* and *parathyroids* were not examined. The heart was enlarged, weighing 37 Gm. and measuring $7 \times 4 \times 3$ cm. The pericardium was negative. The right chambers of the heart were widely dilated. The ductus arteriosus and foramen ovale were closed and the valve orifices and leaflets showed nothing abnormal. Both *coronary arteries* and their major superficial branches were thickened and tortuous, but the thickening was less marked in the posterior artery. The walls of these arteries, on sectioning, contained interrupted circularly disposed calcific plaques and the lumina in many places were markedly decreased. The wall of the left ventricle measured 6 mm. in diameter, the right 3.5 mm. The *aorta* showed irregularly distributed patches of atheromatous thickening. Both *lungs* showed hypostatic congestion in the dependent parts. In addition there were a few scattered small depressed purplish subpleural areas. The other organs showed little of interest.

Microscopic findings. The principal lesions were found in the *arteries of the heart*. The outstanding findings were medial thickening and calcification with fibroblastic proliferation of the intima and narrowing of the lumina, which in some instances had resulted in complete occlusion. The calcareous plaques were circularly disposed but often formed incomplete rings. In many sections the internal elastic lamina were hyalin and thickened, and occasionally in irregular interrupted lengths were encrusted with a granular calcareous coating. In a few small arteries there were intimal atheromatous deposits with degeneration, bordered peripherally by calcareous plaques and overlaid on the luminal surface with fibroblastic proliferation. A small amount of perivascular lymphocytosis was irregularly distributed in the adventitia. In some of the papillary muscles of the left ventricle there were recent infarcts (Fig. 2), with centrally located areas of coagulation necrosis devoid of neutrophils. One section of *aorta* contained a large atheromatous area with thinning of the underlying media. However, the commonest change in the *aorta* was swelling and degeneration of irregularly distributed elastic fibers, some of which showed fine granular bluish encrustings. Large calcareous plaques were also present here and there in the media. The larger branches of the *pulmonary artery* showed a medial thickening and calcification and intimal proliferation of connective tissue similar to that seen in the coronary vessels. In addition to the vascular changes there was a thickening of the alveolar walls which was patchy in distribution and associated with atelectasis and emphysema. The thickening was due to an increased number of round cells; no neutrophils were observed. In some of the areas of interstitial thickening hemorrhage had occurred and erythrocytes and "heart failure" cells were seen in the alveoli. A few of the alveoli were lined with a pink homogeneous membrane obviously derived from exuded serum. The lung picture was that of primary atypical pneumonia. The *spleen* and *gastrointestinal tract* were negative, except

for the medial sclerosis and calcification of arteries as described in the heart. An occasional large hepatic as well as renal artery likewise showed the typical medial calcification and degeneration. The kidney tubules showed fairly extensive parenchymatous degeneration.

Pathologic diagnoses: Generalized arteriosclerosis; medial sclerosis of coronary, pulmonary, gastric, mesenteric, renal and hepatic arteries; atheroma of aorta; coronary stenosis; myocardial infarction, left ventricle; primary atypical pneumonia; parenchymatous degeneration of kidneys.

DISCUSSION

The arterial changes exhibited in all three of our cases were similar to those described in previous reports. This form of arteriosclerosis has been considered as medial by Brown and Richter,¹ Field,² and Hause and Antell,⁴ and as primarily intimal by Stryker.⁵ The process, where well advanced, actually involves all three arterial coats, and might be termed a diffuse arterial sclerosis. No definite etiologic factor has as yet been established for this condition. The presence of a large number of eosinophils in the adventitial and outer medial cellular infiltrates in sections of several of the sclerotic arteries in our first case lends some support to the theory of an allergic background. The occurrence of infantile arteriosclerosis in two siblings as shown in our second and third patients, and its probable occurrence in a previous baby of this family, would seem to implicate a congenital weakness of the elastic tissue in the arterial walls as a possible factor. That this association may not be unusual is suggested by details in cases reported both by Forrer³ and Stryker.⁵ In Forrer's report, a six month sibling of an infant died suddenly, while Stryker remarks that a male sibling of the infant labelled Case 1 in his series expired with similar symptoms.

SUMMARY

In three infants under two months of age, two of whom were siblings, death was caused by coronary arteriosclerosis. The arteriosclerosis was characterized by extensive deposition of calcium.

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NEUROLOGIC SEQUELAE IN MACROCYTIC ANEMIA OF GASTROINTESTINAL ORIGIN FOLLOWING FOLIC ACID THERAPY*

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The incidence of combined system disease in untreated macrocytic hyperchromic anemia other than addisonian pernicious anemia is extremely rare. Peripheral neuritis and mental symptoms are noted more frequently when the general nutrition of the patient is severely impaired. Since the introduction of folic acid therapy, numerous reports have appeared indicating an absence of protection of the central nervous system in patients with pernicious anemia treated with this drug.^{3-7, 11, 12} Recently, Davidson and Girdwood² reported idopathic steatorrhea and mild peripheral neuritis in a patient who was treated with 10 mg. of folic acid daily. The neurologic condition deteriorated during this treatment even though the folic acid was supplemented by thiamine. With discontinuance of the folic acid and substitution of liver extract, the neurologic status improved. A second patient with sprue also developed signs of peripheral neuritis while being treated with 20 mg. of folic acid per day, and the neuritis was completely relieved by the administration of liver extract and other members of the B complex group.

The present report is of interest because of the infrequency of spontaneous combined system disease in patients with macrocytic anemia due to faults in intestinal absorption and the failure of folic acid to prevent the development of this complication.

REPORT OF CASE

In 1926, the patient, a white male, aged 16, was treated at a New York City hospital because of nausea, vomiting, diarrhea and loss of weight. A diagnosis of ulcerative colitis was made. He was found to have a macrocytic hyperchromic anemia and was treated with oral liver extract with clinical and hematologic improvement. In 1928 he developed tuberculous pleural effusion and was treated at a sanatorium. Between 1928 and 1939 the patient suffered numerous attacks of diarrhea, lost weight and developed a moderately severe anemia, which was often of the macrocytic hyperchromic type. He responded moderately well to diet and to oral and parenteral liver therapy. In 1939, at the age of 29, he developed signs of intestinal obstruction and was admitted to the hospital where the terminal ileum and ascending colon were resected. Histologic examination revealed regional ileitis. One year following the operation the patient complained of having large, bulky, soft, yellow stools about 5 times a day and was found to have hyperchromic macrocytic anemia. Injection of liver extract produced an improvement in the blood picture and a reduction in the number and size of the stools. After six months of liver therapy, the patient stopped treatment but returned four months later with the same complaints. At this time liver extract was administered intramuscularly, each injection being followed by

* Read before The Society for the Study of the Blood, New York Academy of Medicine, New York, May 27, 1948. Received for publication, July 16, 1948.

severe urticaria. Occasionally vasomotor collapse occurred. Lamb liver extract produced similar reactions, and oral therapy with liver, ventriculin and Brewer's yeast induced no remissions. From 1941 to 1945 the patient was maintained with blood transfusions and even these were given infrequently since the patient had severe febrile reactions. In November 1945 the red blood cell count was 2.3 million per cu. mm. and the hemoglobin 5.8 Gm. per 100 ml. blood. He was given 50 mg. of folic acid by mouth daily. After four days he reported that his appetite reappeared. At the end of one month the erythrocyte count was 4.5 million, the hemoglobin 9.9 Gm., and he had gained 5 pounds in weight. At the end of two months the red cell count was 4.9 million and the hemoglobin value 11.6 Gm. The patient had then gained 15 pounds, and the stools were better formed and the bowel movements were reduced to two or three per day. At this time the dosage of folic acid was cut to 25 mg. per day and the general well-being of the patient had improved. The total gain in weight was 25 pounds and he was able to return to work. This hematologic and symptomatic improvement was maintained until thirteen months after the beginning of folic acid treatment. In December 1946 he complained of paresthesia in his hands, stating that his fingers became cold, bluish red in color, and that the skin was wrinkled.

A neurologic examination revealed no abnormalities and the patient continued taking folic acid 25 mg. per day. He was referred to the vascular clinic where the disturbance was believed to be nutritional and the patient was given calcium lactate, vitamins A and D, wheat germ and buttermilk, and he was told to stop smoking. In May 1947 the erythrocyte count was 4.4 million per cu. mm. and the hemoglobin level 13.3 Gm. per 100 ml. blood. The patient had not followed the instructions given to him in the peripheral vascular clinic. He now complained of numbness and tightness in his hands, feet and thighs. Neurologic examination revealed a slightly ataxic gait and absence of vibratory sense in the right ankle. However the knee jerks and Achilles reflexes were present, the sense of position of the toes was normal and there were no Romberg's or Babinski's signs. The patient was advised to take 6 capsules of vitamin B complex and 20 mg. of folic acid per day.

The following month the erythrocyte count was 3.9 million and the hemoglobin 16.0 Gm. He complained of numbness of the feet and legs. Ataxia was severe, there was hyperesthesia above the knees and hypesthesia in the legs and feet, the sense of position of the toes was absent, vibratory sense was absent at the ankles and present at the knees, the sense of pain was intact, the knee jerks and the Achilles reflexes were slightly hyperactive, and there was transient ankle clonus and Babinski's sign bilaterally. At this time he was admitted to the hospital. The other significant findings were a red, smooth but painless tongue, and an apparent loss of about 20 pounds in weight. X-ray examination of the gastrointestinal tract showed evidence of regional ileitis. Free hydrochloric acid was present in the gastric juice after histamine.

Skin tests were performed by Dr. Mary Loveless with intraheptol and crude and refined liver extract from various manufacturers. All produced considerable local erythema and swelling. The patient was given 50 mg. of Benadryl (diphenhydramine hydrochloride) t.i.d. and daily injections of increasing doses of liver extract until he was receiving 22 units at one time. There were no local or systemic reactions. The Benadryl was then given at increasing intervals until he was receiving it once every four days. Folic acid therapy was discontinued in July 1947. During his stay in the hospital the red blood cell count was 3.9 million and the hemoglobin varied between 12 and 13 Gm. At the time of his discharge from the hospital he was walking with the aid of two canes. The patient was taught to administer the injections of liver extract (22 units every four days) to himself. He was also given 6 vitamin B capsules by mouth daily and was placed on a high caloric, high vitamin diet.

The erythrocyte count rose to 5.0 million and the hemoglobin to 14 or 15 Gm. and remained constant at these levels. In August the ataxia was moderate, Romberg's sign was present, the sense of position of the toes was gone, the vibratory sense was absent at the ankles but present at the knees, and no paresthesias were present. He required one cane to walk, but in September he discarded the cane, and the only abnormal neurologic findings

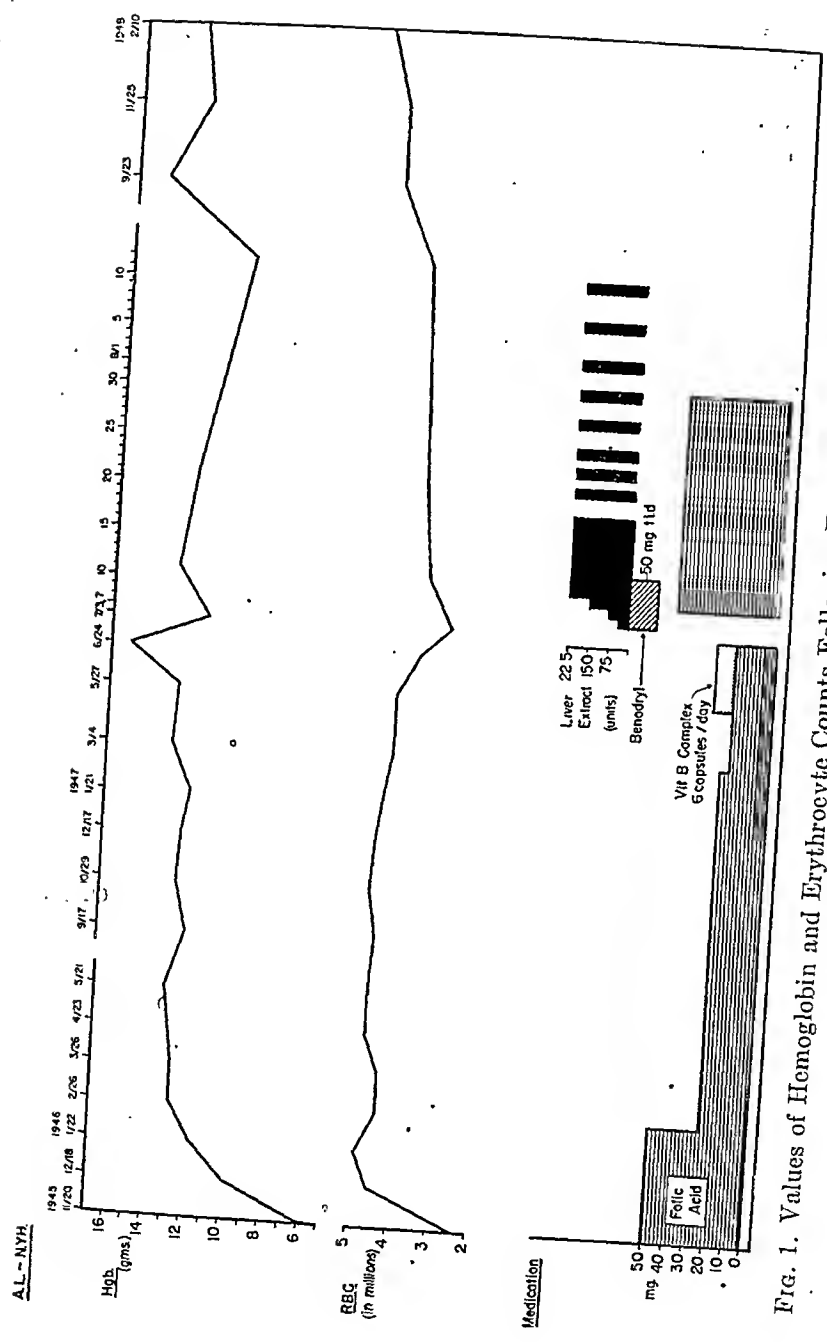


Fig. 1. Values of Hemoglobin and Erythrocyte Counts Following Folic Acid and Liver Extract Therapy

were slight ataxia and diminution of vibratory sense at the left ankle. He had now gained 18 pounds. In November he complained of slight numbness of the soles of his feet with diminution of vibratory sense at the left ankle. In February 1948, his entire neurologic status was normal. At the present time the patient is taking liver extract, 22 units every four days, and 6 vitamin B complex capsules daily.

DISCUSSION

Two explanations have been offered for the development of neurologic sequelae in patients with pernicious anemia treated with folic acid. Ross⁷ has suggested that folic acid, which contains a glutamic acid radical, may interfere with the normal metabolism of glutamic acid in the nervous system in a competitive manner similar to the action of para-aminobenzoic acid in inhibiting the activity of the sulfonamide drugs. Davidson and Girdwood² suggested that there may be an antagonism between members of the vitamin B complex groups since patients showing vitamin deficiency, when treated with one member of the vitamin B complex, will develop signs of deficiency of another member. The clinical evidence for the latter view has been demonstrated in pellagra and riboflavin deficiency.^{1, 8-10}

SUMMARY

A patient with macrocytic hyperchromic anemia, attributable to faulty intestinal absorption, was successfully treated with liver extract to which he became sensitive. Treatment with folic acid for thirteen months was followed by the development of peripheral neuritis and combined system disease. Successful desensitization to liver extract and treatment with this drug resulted in complete remission of the neurologic disease and return to health.

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CLINICOPATHOLOGIC CONFERENCE*

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Dr. T. K. Leonard, resident in internal medicine. A white married woman, aged 36, first entered the Iowa Methodist Hospital on September 1, 1937, complaining of hemorrhoids and a draining sinus near the anus for eighteen years, anorexia for seven weeks and debility for two weeks.

Early in 1937 after a dental extraction, she developed chills and fever followed by extensive ulcerative inflammation of the gums and pharynx lasting two months. This condition had improved prior to admission; however, her appetite was poor, and for two weeks she had remained in bed because of weakness. She related that in 1931 she had developed arthritis which receded after about two years. In 1933 she had had a severe pain in the left lower abdominal quadrant, as if "something broke". At that time a painless mass was noted beneath the left costal margin. She had borne 8 children and had had four miscarriages. Her menstrual periods had always been regular but scanty.

On admission to the hospital, she was afebrile, well developed and slightly obese. There was extensive pyorrhea, and the posterior pharyngeal wall was injected. The blood pressure was 98/66. The findings in the lungs and heart were normal. Examination of the abdomen revealed "grade 2 enlargement of the spleen". A fistula-in-ano had its opening one inch superior to the anal orifice.

A scheduled hemorrhoidectomy and fistulectomy was cancelled when it was discovered that the hemogram revealed erythrocytes 2,980,000 per cu. mm., hemoglobin 60 per cent, leukocytes 1600 per cu. mm. with lymphocytes 77 per cent, monocytes 10 per cent, neutrophils 12 per cent and eosinophils 1 per cent. No immature cells were found in the blood smears. Gastric analysis showed free hydrochloric acid. The blood Kline and Kahn flocculation tests were negative.

Throughout most of a seven week period of hospitalization she showed a slight afternoon elevation of temperature. There were episodes of epistaxis, frontal headache and severe cramping pain in the lower midabdomen. Despite administration of pentnucleotide, yellow bone marrow, liver extract and 500 cc. of blood, the blood picture remained unaltered, and she was discharged unimproved.

She entered another hospital early in 1938, complaining of weakness and fatigue. She was found to have an ulcer of the roof of the mouth and enlargement of the spleen which reached to the anterior superior iliac spine. The blood counts were similar to those previously reported. Blood smears showed "mild diminution of platelets", and a biopsy of the sternal marrow revealed

* Received for publication, June 18, 1948.

hyperplasia of cellular elements. Blood cultures were negative. Fecal analysis was negative for occult blood. The total protein was 7.2 Gm., albumin 3.8 Gm., globulin 3.4 Gm., and serum bilirubin 1 mg. per 100 ml. The prothrombin time was 63 per cent and the basal metabolic rate plus 6 per cent.

She developed a large, deep, poorly defined area of ulceration with local inflammation over the right greater trochanter. After x-ray therapy, a crust began to form; nevertheless, the wound failed to heal for many months. The sternal biopsy wound also continued to drain, and *Pseudomonas aeruginosa* was recovered from it. She received 6 transfusions and had a chill during or following each one. Two hundred roentgens of radiation were applied over the spleen, and her leukocyte count fell to 800 per cu. mm. She was discharged with a tentative diagnosis of lymphoma or aleukemic leukemia.

She then began to improve in strength and to gain weight and was able to work until January 1940, when she became dyspneic and developed pain under the left breast. Later, she was confined to bed at home for a month with a sore on her right leg. In 1942 she miscarried in the third month of a pregnancy, and a foul vaginal discharge persisted for four months. In March 1944 she developed severe painful swelling of the right leg and again was in bed for a month. In August 1944 she noticed persistent burning and frequency of urination; the urine had a foul odor. In December 1944 she developed pain, swelling and tenderness of her joints and was again forced to go to bed. In December 1945 an attack of laryngitis lasted for six weeks. She had been quite obese, but now began to lose weight. Early in 1946 a sore appeared on her right leg, and a large tender lymph node was present in the neck.

She was readmitted to the Iowa Methodist Hospital in June 1946 because of purulent otitis media. Her leukocyte count was 800 per cu. mm., erythrocyte count 2,360,000 per cu. mm. and hemoglobin 5.4 Gm. per 100 ml. She was found to be group O, Rh-negative, but despite the administration of compatible blood, she had a severe chill and fever following transfusion. She improved rapidly with penicillin therapy and was dismissed.

She re-entered the Iowa Methodist Hospital two months later complaining of cough, blood-streaked sputum, pain under the right scapula and fever of four weeks' duration.

Physical examination revealed a pustular infection of the face and back. Over the apex of the right lung, there were noted dullness, increased transmission of the whispered voice and dry rales. A large ill-defined mass was palpated in the left upper portion of the abdomen; its lower edge lay below the level of the anterior superior iliac spine and was smooth and round, and its upper part seemed to disappear beneath the left costal margin. Pelvic and rectal examinations were negative except for a fistula-in-ano.

The admission hemogram showed hemoglobin 7.6 Gm., erythrocytes 2,600,000 per cu. mm., leukocytes 3700 with the following differential count: lymphocytes 56, neutrophils 7, stab cells 22, monocytes 9, eosinophils 4, basophils 1 and plasma cells 1 per cent. A blood smear was reported as showing "Normochromic microcytic anemia characterized by spherocytosis and polychromato-

philia, rather pronounced neutropenia, moderate left shift with minimal toxic changes of the neutrophils; blood platelets present in moderate numbers". The platelet count was 132,000 per cu. mm. (slide method), reticulocyte count 7.9 per cent, coagulation time (slide method) four and one-half minutes, bleeding time six minutes and prothrombin time (Smith) 100 per cent. An erythrocyte fragility test showed hemolysis beginning at 0.46 per cent and complete at 0.38 per cent. In the control test, hemolysis began at 0.42 per cent and was complete at 0.34 per cent. The serum bilirubin was less than 0.25 mg. per 100 ml. The urinalysis was negative.

An x-ray film of the chest showed a rarefied area 2 cm. in diameter, overlying the anterior end of the first rib, having the appearance of a cavity. The radiologist suggested that the lesion might be tuberculous. However, although the tuberculin test was positive, sputums and gastric washings contained no acid-fast bacilli. Smears and cultures of sputum showed a mixed bacterial flora and no fungi. An abscess developed over the right ischium, and from it *Klebsiella pneumoniae* was recovered.

A roentgenogram of the chest five weeks later revealed marked improvement in the right apical lesion. She was transferred to another hospital where an operation was performed.

CLINICAL ANALYSIS

Dr. Arthur G. Lueck, internist. In going over the case history, several diseases come to mind; in fact, the list is quite long. I will try to narrow it down to five of the most likely possibilities. The first of these is Banti's syndrome. If we are to accept Banti's disease as an entity, it may be defined as follows: A chronic disease of unknown origin, primary in the spleen, characterized by splenomegaly, anemia, leukopenia, a tendency to gastric hemorrhage, increased formation and destruction of erythrocytes and later by cirrhosis with ascites and jaundice. It has been suggested that the etiology is hypertension of the portal system. Much similarity between Banti's syndrome and this case is apparent.

A second strikingly similar disease comes to mind. Kala-azar is an infectious disease characterized by splenomegaly, hepatomegaly, continuous irregular fever, anemia, leukopenia and loss of weight and strength. A parasitic organism, *Leishmania donovani*, is present in the peripheral blood and the cells of the reticulo-endothelial system. For a diagnosis of kala-azar, the patient must have journeyed to Asia (particularly India or China), one of the Mediterranean countries or South America. A few cases have been recorded in the United States, but all were contracted by travel or residence in countries where kala-azar is endemic.

Gaucher's disease is a rare condition characterized by chronic anemia, a hemorrhagic tendency and marked splenomegaly. Peculiar large lipid-containing cells are found in the spleen as well as in the liver, bone marrow and lymph nodes.

The findings of spherocytosis and of increased erythrocyte fragility, along with anemia and splenomegaly, call attention to the possibility of familial

hemolytic jaundice. This disease seems to be due to increased fragility of congenitally spherocytic erythrocytes. Chronicity, splenomegaly and anemia are its outstanding features. The marrow is hyperplastic as in this case. However, absence of icterus and leukocytosis, characteristic of hemolytic jaundice, diverts our attention from this condition as a probable diagnosis.

In 1939 and 1942, Wiseman and Doan^{4, 5} reported a newly recognized granulopenic syndrome, caused by excessive splenic leukolysis and successfully managed by splenectomy, termed "primary splenic neutropenia". This is the fifth condition on our list. As a matter of fact, at least three diseases are apparently produced by excessive splenic destruction of blood elements. In the first of these three, thrombocytopenic purpura, there is excessive platelet destruction; in the second, familial hemolytic icterus, congenitally fragile erythrocytes are excessively phagocytosed, and in the third, primary splenic neutropenia, granulocytes are excessively destroyed. In all three of these diseases, splenectomy produces great improvement. Primary splenic neutropenia is characterized by fever, pain over the splenic region, splenomegaly, hyperplasia of the bone marrow, mild thrombocytopenia and, as an outstanding finding, persistent granulocytopenia.

A fourth disease, described by Doan and Wright,³ has been added to the foregoing triad. It is a condition in which there is a reduction of all of the main formed elements of the blood. It is known as primary splenic panhematopenia. Characteristically, leukopenia is the most persistent feature; anemia is also persistent, though moderate; thrombocytopenia is usually clearly demonstrable. Leukopenia, anemia and thrombocytopenia are all relieved by splenectomy. This case could be interpreted as primary splenic panhematopenia if it were not for lack of evidence of definite thrombocytopenia. The platelets were recorded on one occasion as "moderate in number", on another occasion as "mildly diminished" and on a third occasion numbered 132,000 per cu. mm.

In summary, the chronicity, splenomegaly, hepatomegaly, anemia and neutropenia suggest a diagnosis of Gaucher's disease. These findings, plus fever and chills, suggest the possibility of kala-azar. Add thrombocytopenia and marrow hyperplasia and one thinks of Banti's syndrome. The further addition of recurrent infections calls to mind the possibility of splenic neutropenia, and, if the thrombocytopenia were more pronounced, one would be tempted to make a diagnosis of primary splenic panhematopenia. On the other hand, two features are characteristic of hemolytic jaundice, namely spherocytosis of erythrocytes and increased erythrocyte fragility.

Taking into consideration all of the factors, some significant and some misleading, my diagnosis is primary splenic neutropenia.

DIAGNOSES

Clinical diagnosis: Primary splenic panhematopenia.

Dr. Lueck's diagnosis: Primary splenic neutropenia.

Postoperative diagnosis: Primary splenic panhematopenia.

PATHOLOGIC FINDINGS

Dr. R. F. Birge, clinical pathologist. The clinical diagnosis, made before the patient went elsewhere for further study, was primary splenic panhematopenia. This interpretation differs from Dr. Lueck's only because the case history left him uncertain that there was definite thrombocytopenia.

We withheld from Dr. Lueck one observation, namely, the result of a so-called "adrenalin test". This test has been proposed by Doan and Wright³ on the premise that in splenic panhematopenia the enlarged spleen sequesters and destroys excessive numbers of erythrocytes, leukocytes and platelets. When adrenalin is given in such cases, the spleen will contract, sequestered elements will be liberated from it and the counts of all the cells will increase.

Dr. Glomset had wondered whether or not those who had studied the patient in the past had been correct in considering the mass in the left upper quadrant as spleen. Could it not be a big kidney or a retroperitoneal neoplasm? When adrenalin was given, the mass decreased more than 4 cm. in diameter within a few minutes. This observation left little doubt that the mass definitely was spleen. Within fifteen to thirty minutes after the administration of adrenalin, the hemoglobin increased from 9.0 to 11.2 Gm. per 100 ml., the erythrocyte count from 3,390,000 to 4,160,000 per cu. mm., the leukocyte count from 900 to 2400 per cu. mm. and the platelet count from 36,000 to 68,000 per cu. mm.

A short time later, a sternal puncture, made elsewhere, showed evidence of normoblastic hyperplasia and left shift of the myeloid series of cells. Thereafter, splenectomy was done. The spleen weighed 1900 Gm. Its general architecture was preserved, but the follicles were small and widely scattered. The sinusoids were rather prominent, but in some areas were barely discernible because of thickening of their walls with considerable proliferation of the reticulo-endothelial tissue about them. There seemed to be a moderate increase of eosinophils through the pulp. There were small collections of brown pigment, presumably old blood pigment, usually contained within macrophages. I felt that the findings in the marrow and spleen were consistent with splenic panhematopenia.†

However, the proof of the correctness of the diagnosis would seem to lie in the postoperative course. It is now about one year after operation. If our diagnosis is correct, she should by now show considerable improvement, for you will remember that she was very sick with frequent infections and had been intermittently bedridden for many years.

DISCUSSION

Dr. Daniel A. Glomset, internist. This patient had been under the care of several excellent physicians, and all had thought she was suffering from lymphoma or an aleukemic phase of myelogenous leukemia. I am not a hematologist, but I did not believe an aleukemic phase of leukemia could last as long as thirteen years. Dr. Birge was conversant with Doan's idea that the spleen

† Marrow smears and portions of the spleen were obtained for study through the courtesy of Dr. Charles R. Watkins and of Dr. Albert C. Broders, Rochester, Minnesota.

may sequestrate and destroy elements of the blood at such an accelerated rate that anemia, leukopenia and thrombocytopenia will occur, bone marrow hyperplasia notwithstanding. He suggested that a splenectomy might be of benefit.

She had an uneventful postoperative course, and I have seen her several times since. She has had no more infections. She had been bedridden for two years prior to surgery. Now she is able to do most of her housework, walk downtown and attend social meetings. Her erythrocyte count has risen from 2,600,000 to 5,300,000 per cu. mm., the hemoglobin from 7.6 to 14.0 Gm. per 100 ml. and the leukocyte count from 3700 to 7000 per cu. mm.

The experience has been that most of such patients are not completely well postoperatively, but are restored approximately to ninety per cent of well-being. This patient still suffers from atrophic arthritis, but I do not believe that this condition is a sequela of splenic panhematopenia. She is still somewhat weak. Although she does her ironing, she does it sitting down. Then, too, her blood picture is not completely normal, since, despite a normal total leukocyte count, the differential count remains abnormal. Before surgery she had 56 per cent lymphocytes, and nine months after surgery she had 63 per cent lymphocytes. Thus, although her total polymorphonuclear count has about doubled, the proportion of lymphocytes to granulocytes remains the same. The explanation for this phenomenon remains obscure.

Dr. John C. Parsons, internist. What is the explanation for the appearance and disappearance of pulmonary cavitation?

Dr. Glomset. Many sputums were negative for acid-fast bacilli. The lesion in the right apex had almost disappeared by the time the woman was dismissed and was presumed to be a nonspecific pulmonary abscess.

Dr. Allan Phillips, radiologist. The process must have been an abscess. Pulmonary abscesses not infrequently heal rapidly, particularly under the influence of chemotherapy.

Dr. Birge. The outstanding feature of such cases as this is the occurrence of numerous and varied infectious processes. Concerning her arthritis, it is at least not a unique complication, for, recently, we have observed a case of splenic neutropenia associated with atrophic arthritis and skin ulceration. Of course, when leukopenia and splenomegaly are accompanied by arthritis, we are confronted with the concept of Felty's syndrome. Perhaps some cases, believed in the past to have been examples of Felty's syndrome, were instances of splenic neutropenia complicated by rheumatoid arthritis.

In closing, may I add that it has not been the purpose of this discussion to debate theories of etiology and pathogenesis of so-called "hypersplenism". However, it should be recognized that Dameshek^{1,2} has disputed the theory of Doan and Wright, that the major process is one of sequestration, with destruction by phagocytosis, in the spleen, of formed elements of the blood. He believes that, at least in idiopathic thrombocytopenic purpura, an unusual inhibitory effect is exerted upon the marrow by the overactive spleens of these patients. Interesting to me has been the occasional observation of erythrophagocytosis in the peripheral blood in familial hemolytic icterus in crisis and in

the bone marrow in splenic panhematopenia; I have not observed this phenomenon in other anemias. There seems to be strong evidence that excessive phagocytosis is at least one factor in production of the anemia observed in this group of diseases.

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BOOK REVIEWS

Pathology of Tumours. By R. A. WILLIS, D.Sc., M.D., F.R.C.P., Sir William H. Collins Professor of Human and Comparative Pathology, Royal College of Surgeons, London, Formerly Pathologist to the Alfred Hospital, Consultant Pathologist to the Austin Hospital for Chronic Diseases and Lecturer on the Pathology of Tumours in the University of Melbourne, Australia. 992 pp., 500 figs. \$20.00. St. Louis: The C. V. Mosby Company, 1948.

The author, a veteran in the field of oncology, proves himself to be a profound student and a brilliant writer. He is frank, critical and sensible in his appraisal of widely accepted views. Originality, clearness of expression, first hand observations and a willingness to depart from time honored but badly frayed dogma delight the reader and win respect for the writer. Although drawing heavily upon two excellent sources of material, *Nicholson's Studies on Tumour Formation*, published in 1921 to 1933, and the author's previous book, *The Spread of Tumours in the Human Body*, published in 1934, he has shown no partiality to British authors or publications. Part I is a comprehensive and fundamental presentation of the subject, covering two hundred pages, bringing many controversial general aspects up to date and providing refreshing and profitable reading.

The reviewer's remarks up to this point might appear to be uncritical and somewhat extravagant, but actually they represent a fair appraisal of the book. One might be inclined to disagree with some of the viewpoints expressed, but the issues would be of minor importance.

In Part II some of the chapters are admittedly stronger than others, the chief fault being that some of the subjects could have been developed more exhaustively. The author does not completely clarify the subject of lymphoid tumors, but contributes a valuable general discussion and expresses a modern viewpoint. The absence of a highly satisfactory classification of neoplasms is disappointing; it seems high time that one was forthcoming.

The section on experimental carcinogenesis is excellently written, the work done in this country and the recent brilliant contributions of the author's fellow countrymen being well represented. His interest in comparative pathology is a valuable asset throughout the book, especially with tumors in other species, promoting a better understanding of those occurring in human beings. The vigorous emphasis which he has placed upon the multicentric origin and continuous independent development of tumors, and also the intimate relationship between hyperplasia and neoplasia, seems highly appropriate.

The chapters dealing with salivary gland tumors and adamantinomas are valuable and refreshing. The discussion of neurofibromas and neurilemmomas is excellent and the stressing of their inter-relationship most appropriate, although Stout would criticize spelling of the latter of the two nouns with three m's. The paragangliomas and parathyroid tumors could have been more thoroughly dealt with, and the chapters on bone tumors and urogenital neoplasms could have been more masterful.

The subject material is well arranged. There are five hundred black and white illustrations, most of which are excellent photomicrographs.

The author bores courageously into the heart of the subjects, which he discusses with high regard for the axiom, "We are not bound to accept,—indeed, we are properly bound to reject—a proposition that is not clear and distinct".

Detroit

OSBORNE A. BRINES

Bergey's Manual of Determinative Bacteriology. Ed. 6. By Board of Editor-Trustees: ROBERT S. BREED, New York State Experiment Station (Cornell University), Geneva, New York; E. G. D. MURRAY, McGill University, Montreal, Province Quebec, Canada; A. PARKER HITCHENS, University of Pennsylvania, Philadelphia, Pennsylvania. Assisted by sixty contributors. 1529 pp. \$15.00. Baltimore: The Williams & Wilkins Company, 1948.

To the average garden variety of bacteriologist the publication of a new edition of Bergey's Manual is an event of importance which he regards with interest mixed with feelings of trepidation. His interest is stimulated by the knowledge that "Bergey" constitutes the outstanding compendium of bacterial species which has a utilitarian as well as a scientific value. His feeling of trepidation results from the sure knowledge that each edition of "Bergey" introduces changes in bacteriologic nomenclature, together with rearrangements of genera and species that are unsettling to his comfortable sense of stability. Unless he has followed developments in taxonomy, the changes are difficult for him to understand and even more difficult to accept.

Our sympathies are with the bacteriologist in his desire for nomenclature that will stay put. However, while stability of nomenclature is desirable for the student, the investigator, and the routine worker, it is impossible of attainment at the present imperfect state of bacteriologic taxonomy. It has been pointed out by Wilson and Miles that the criteria that have determined the classification of bacteria would not satisfy the systematist in any other branch of biology. When one considers that systematic bacteriology is based in part on morphology, on physiology, on pathogenicity and antigenic relationships of bacterial cells, it is obvious that changes will be frequent in years to come as taxonomists strive for a more logical system.

The Committee on Manual of the first edition of Bergey's Manual, published in 1923, made plain that their efforts constituted a progress-report. The Board of Editor-Trustees of the Sixth Edition likewise state frankly that the Manual is an effort to keep pace with rapidly expanding knowledge. The fact that the Manual is widely accepted as an authoritative source, proves that it has been of value in both an academic and utilitarian sense.

Some extent of the changes that have occurred since 1923 may be realized by the fact that the First Edition contained descriptions of 94 genera and 832 species, while the new publication has descriptions of 176 genera and 1630 species. Medical bacteriologists and virologists will be interested particularly in the new Supplements I and II, under which are outlined in detail the Orders Rickettsiales and Virales. It will be a surprise to many to find the familiar common names of bacteriophages and viruses disguised by scientific nomenclature in these supplements. Certain generic names that were a part of the literature have disappeared in the interests of taxonomy. Of importance to medical bacteriologists is the abolition of the generic name *Staphylococcus* and the incorporation of the staphylococci under the genus *Micrococcus*. The familiar designation *Eberthella* has disappeared and *E. typhosa* now appears as *Salmonella typhosa*. The generic name *Listerella* has been changed to *Listeria*, while an entirely new genus *Moraxella* has been inserted under the Tribe Hemophilae.

Specialists in various fields of bacteriology will not be satisfied with some of the exclusions and insertions. For example, it is difficult to understand the retention of an imperfectly described species such as *Shigella gintottensis* and the exclusion of the mannitol-negative dysentery organisms described by Large and Sachs.

Imperfect though it may be, the new Bergey's Manual is without doubt the best classification of bacteria available. Every worker in the field of bacteriology owes the editors and specialists who made the volume a debt of gratitude.

Lansing, Michigan

G. D. CUMMINGS

The 1947 Year Book of Pathology and Clinical Pathology. Pathology edited by HOWARD T. KARSNER, M.D., Professor of Pathology, Director of the Institute of Pathology, Western Reserve University, Cleveland. Assistant Editor, HERBERT Z. LUND, M.D., Assistant Professor of Pathology, Western Reserve University, Cleveland. Clinical Pathology edited by ARTHUR HAWLEY SANFORD, M.D., Professor of Clinical Pathology, University of Minnesota, (The Mayo Foundation); Senior Consultant, Division of Clinical Laboratories, Mayo Clinic. 558 pp., 103 figs. \$3.75. Chicago: The Year Book Publishers, Inc., 1948.

The Year Book of Pathology was inaugurated in 1940 as one of 14 Practical Medicine

Series of Year Books under the joint editorship of Karsner and Hooker. It was discontinued in 1941, due to conditions of the war period. The resumption has been eagerly awaited by many who have learned to appreciate the valuable book.

The changes in the editorial staff are indicated in the opening paragraph of the review. The readers of this journal will welcome the change of the name of the book from *Year Book of Pathology and Immunology* to *Year Book of Pathology and Clinical Pathology*. They will also delight in seeing the editorship of clinical pathology in the hands of Arthur H. Sanford, the past master of our specialty.

The 312 pages of the section on pathology are devoted to general pathology, tumors, to diseases of the cardiovascular, hemopoietic, respiratory, alimentary, urinary, female genital, osseous, internal secretory and nervous systems, and include a chapter on miscellaneous subjects and one on technical methods.

The 220 pages of the section on clinical pathology deal with hematology, clinical chemistry, bacteriology, viruses, mycology, allergy, parasitology, gastric analysis, urinalysis, cytology and apparatus.

The presentation consists of abstracts with numerous pointed editorial comments. The difficult job of selection was accomplished most commendably. There is something significant on every page. The abstracts are well done, clear and brief, but include the essential points of the original article.

Readers will welcome concise reviews on the following important and timely subjects which were prepared on request: cytology of vaginal smears (Allan and Hertig), invasiveness of cancer (Coman), lymphatic and hemopoietic organs (Smith and Custer), nervous system (Globus), pulmonary embolism (Holman), and bones (*l'affe*).

No pathologist will want to be without this handy and very helpful digest of developments in pathology and clinical pathology during 1947.

Chicago

I. DAVIDSOHN

Practical Bacteriology, Hematology, and Parasitology. Ed. 10. By E. R. STITT, M.D., Ph.M., Sc.D., LL.D., Rear-Admiral, Medical Corps, and Surgeons General, U. S. Navy, Retired, Formerly Head of the Department of Tropical Medicine, U. S. Naval Medical School; PAUL W. CLOUGH, M.D., Assistant Professor of Medicine, Johns Hopkins University, Associate Professor of Medicine, University of Maryland; SARA E. BRANHAM, M.D., Ph.D., Sc.D., Senior Bacteriologist, National Institute of Health, Chairman, Laboratory Section, American Public Health Association, 1946-1947; and CONTRIBUTORS. 991 pp., 765 illus., 7 plates in color. \$10.00. Philadelphia: The Blakiston Company, 1948.

This reviewer has worked with Stitt's book since it first appeared in 1908 and has looked upon the appearance of each new edition as a notable event; in his opinion this, the tenth, is all of that. It is a pleasure to review a book of this order.

As in previous editions, and as the title indicates, greatest emphasis has been placed on Parts I, II, and III which deal with Bacteriology, Hematology, and Parasitology respectively.

The section on Bacteriology has been revised, almost entirely rewritten and brought up to date by Dr. Branham, co-editor, who replaces Dr. Mildred Clark Clough, to whose memory the book is dedicated. As evidence of modernity it might be mentioned that *Eberthella typhosa* of the previous edition is now *Salmonella typhi*. So much has been added that it is impossible to treat each subject adequately but special mention must be made of the chapter on Medical Mycology rewritten by Lt.-Comdr. Robert J. Goodlow of the Naval Medical School and especially of the part describing the dermatophytes.

Part III on Parasitology by three new contributors, Comdr. Elmer M. Bingham, Comdr. Trenton K. Ruebush, and Lt.-Comdr. William J. Perry is outstanding. It has been rewritten and much new material added. The scheme of description generally follows this order: Classification, Morphology, Life Cycle and Prophylaxis, Clinical Illness and Diagnosis. Laboratory techniques are, of course, given in detail. There is a profusion of new illustrations depicting the life cycle of many of the parasites and of others, the structure.

Much has been added to Part II on Hematology although the revision does not seem to be as extensive as the two previously discussed sections.

In Part IV entitled Clinical and Pathological Examinations of Body Fluids and Organs, methods for the examination of urine, feces, sputum, gastric contents, chemical examinations of the blood and other fluids are given in proper detail. Chapter 44 of this part on Vitamins as Specific Food Factors has a select bibliography appended. One could wish that other subjects had been similarly treated. Names of authors with the year of their contribution are seen frequently but the name of the journal is missing, thus making it necessary to consult one of the large indices.

This treatise, excellent as always, largely rewritten, greatly enlarged, well illustrated, thoroughly up to date and a fine example of book-making, cannot be recommended too highly. The editors, contributors and publishers are to be congratulated for a superb production.

Rochester, New York

W. S. THOMAS

Hemostatic Agents. With Particular Reference to Thrombin, Fibrinogen and Absorbable Cellulose. By WALTER H. SEEGER, M.S., Ph.D., Professor of Physiology, Wayne University College of Medicine, and ELWOOD A. SHARP, M.D., Sc.D., Director, Department of Clinical Investigation, Parke, Davis and Company, Lecturer, Department of Medicine, Wayne University College of Medicine. 131 pp., 27 figs., 9 tables. \$4.75. Springfield, Illinois: Charles C Thomas, 1948.

In recent years several products possessing hemostatic properties have been developed commercially. Drs. Seegers and Sharp have gathered together the various clinical experiences with these products (thrombin, fibrinogen, oxidized cellulose, fibrin foam and gelatin sponge) and have made it the material for a monograph of 96 pages plus 370 references. They have included their own significant part in the development of these agents together with a theoretical presentation of the coagulation mechanism.

The monograph contains much interesting material, but at times lacks critical judgment. In a work devoted to hemostatic agents, one should expect a short introduction dealing with the general aspects of hemostasis, including references to the vascular response to injury, to the role of platelets and to the vascular hormones such as epinephrine and histamine. Instead one finds a rambling presentation of blood clotting which conspicuously omits important advances made during the past five years but includes a discussion of the hemorrhagic diseases of the newborn, a facsimile of Dam's first statements that led to the discovery of vitamin K, a comparison of the potency of heparins of various species and many similar topics, which though important in themselves, are hardly germane to the primary objective as specified in the title. The chapter on thrombin might well have included a statement of the valuable pioneer work of Mellanby in preparing this agent in pure form. In presenting the practical applications of thrombin and the other agents, little mention is made of their possible limitations. The authors fail to state what can be expected from these agents in such conditions as hemophilia where the need for effective hemostats is greatest. The book contains attractive color plates and the format is outstanding.

Milwaukee, Wisconsin

ARMAND J. QUICK

Identification of Tumors. By N. CHANDLER FOOT, M.D., Professor of Surgical Pathology, Cornell University Medical College, Surgical Pathologist to New York Hospital. 397 pp. 241 figs. \$6.00. Philadelphia: J. B. Lippincott Company, 1948.

This book serves as a useful reference for students, surgeons and practitioners who may desire a systematic arrangement of essential information relating to neoplastic diseases. All historical and theoretical data have been eliminated. The text affords an adequate working concept of the salient gross and microscopic features of tumors of general distribution as well as those which are peculiar to organs. In essence, the book is a compendium of practical information. Photomicrographic illustrations of most tumors are profusely supplied and, in general, are of good character. The preferred name of each tumor is presented

together with its less familiar or apposite synonyms. The tissue of origin and the analogue of the tumor are stated. The usual age and sex of the patient is indicated and important signs and symptoms are briefly noted. Some improvement in usefulness of text could have been attained by a more complete indication of the clinical significance and the response of given tumors to irradiation therapy.

A very brief chapter deals with technical methods of value in the diagnosis of tumors.

The book should serve all who wish to refresh their knowledge of neoplasms before taking specialty board examinations.

Detroit

ARTHUR L. AMOLSCH

Practical Methods of Biochemistry, Ed. 5. By FREDERICK C. KOCH, Frank P. Hixon Distinguished Service Professor Emeritus of Biochemistry, University of Chicago, Director of Biochemical Research, Armour and Co., Chicago; and MARTIN E. HANKE, Associate Professor of Biochemistry, University of Chicago. 419 pp., 51 tables. \$2.50. Baltimore: A William Wood Book, The Williams & Wilkins Company, 1948.

The new edition of Koch's *Practical Methods of Biochemistry*, like previous editions, is an invaluable text for biochemical procedures. While this manual is intended for medical students, since the quantitative aspects of physiologic chemistry have been particularly emphasized, both clinicians and biochemists should include it in their libraries as a standard reference. However, the scope of methods precludes its use in its entirety simply as a laboratory manual for a course in medical biochemistry. The principal revisions in this edition consist in an expansion of manometric methods and the addition of microbiologic and colorimetric methods for vitamins and amino acids.

It is to be regretted that the manual lacks a more comprehensive treatment of photo-electric principles as well as their application to the newer biochemical methods. Some of the more recent chemical methods for blood constituents are likewise absent. Revisions in some of the older terminology could have been profitably made.

The format, printing and binding are good. On the whole, the manual is an excellent one and should continue to serve as a reference text for biochemical procedures.

Detroit

ARTHUR H. SMITH

A Symposium on the Use of Isotopes in Biology and Medicine. By HANS T. CLARKE, HAROLD C. UREY, GLENN T. SEABORG, PAUL C. AEBERSOLD, ALFRED O. NIER, CHARLES C. CORYELL, MARTIN D. KAMEN, DONALD B. MELVILLE, DAVID B. SPRINSON, HARLAND G. WOOD, KONRAD BLOCH, DAVID M. GREENBERG, I. L. CHAIKOFF, JOSEPH G. HAMILTON, BYRON E. HALL, SAUL HERTZ, WILLIAM F. BALE, JAMES J. NICKSON, FARRINGTON DANIELS. 445 pp., illustrated. \$5.00. Madison, Wisconsin: The University of Wisconsin Press, 1948.

Following an introductory chapter by H. T. Clarke giving the historical background of tracer experimentation with isotopes, seven chapters by H. C. Urey, G. T. Seaborg, P. C. Aebersold, A. O. Nier, C. D. Coryell, M. D. Kamen and D. B. Melville, respectively, cover the preparation of isotopes for tracer work, together with methods of detection and measurement. The statement of C. D. Coryell (p. 101) that no general textbook yet exists for either nuclear chemistry or general radiochemistry, may be true, but these expositions, together with the extensive and well-selected bibliographies covering the theoretical as well as the practical aspects, constitute an excellent substitute for such a text and could well be used for that purpose. A knowledge of fundamentals of physics is essential.

D. B. Sprinson, H. G. Wood and Konrad Bloch contribute the chapters on protein, carbohydrate and fat metabolism. In spite of the statement by Wood (p. 240) that, on account of space, many important investigations have been omitted from consideration, one feels that a very broad yet intensive survey of the fields has been presented. At the same time the authors have succeeded in selecting typical investigations with the several isotopes, illustrating the general types of research which are concerned with them.

A series of tracer studies on metabolism of other elements,—mineral elements by D. M.

Greenberg, iodine by I. L. Chaikoff and A. Taurog, and some other elements by J. G. Hamilton, all presenting metabolism in some pathologic as well as normal conditions,—serve as introduction to the two later chapters dealing with the therapeutic use of radioisotopes. These are presented by B. E. Hall on the use of radiophosphorus in various blood diseases, and by Saul Hertz on treatment of the thyroid diseases by means of radioactive iodine.

A highly useful chapter on health hazards in the use of radioactive isotopes, contributed by Wm. F. Bale, defines terms applied to measurements of radioactivity and gives detailed examples of methods of computing dosages from emitters of various types of radiations. The article by J. J. Nickson, on measures for the protection of personnel and property, is also highly practical and serves as a wholesome warning to the uninitiated. The chapter by H. C. Urey, on international aspects of atomic energy, offers the suggestions of one of the men who have been thinking long and intensively upon this subject which should be a matter of serious consideration to all scientists. The moderating ideas of Farrington Daniels on the development of atomic energy is also a well-selected topic.

In general, it may be said that this Symposium presents, for beginners, a broad survey of the field of Isotopes in Medicine which is sufficiently detailed to serve as a text, while for the more advanced investigator it offers an opportunity to keep abreast of recent developments in a wide range of subjects for which isotopes are now being used.

Philadelphia

GRACE MEDES

Manual de Enfermedades Infecciosas. Volumen 1. Infecciones Bacterianas y Rickettsiosis.

By C. IONESCU-MIHAESTI and M. CIUCA. Spanish Translation by M. Cano of the Faculty of Medicine, Barcelona, Spain. 61 pp., 20 Pesetas, Barcelona, Spain: Ediciones B.Y.P. Calle Calabria 66, 1948.

This manual of infectious diseases stems from a project initiated in 1915 in Bucharest in the completion of a military manual for the Romanian Army. It was revised and amplified in 1939 and again in 1944 for a similar purpose by Ionescu-Mihaesti and M. Ciuca of the Cantacuzene Institute, Bucharest, Roumania.

It is no more than an outline embracing the bacterial, spirochaetal and rickettsial diseases. On the whole, but a single page is devoted to each disease. This brevity necessitates outline form and the following points are considered under the heading of each disease: the pathogenic agent, its portal of entry, clinical symptoms, prognosis, mode of spread, laboratory diagnosis, treatment and prophylaxis.

Being a manual for Eastern Europe, it includes material on some of the following diseases which in our region receive little attention: anthrax, botulism, pond fever (*Leptospira grippotyphosa*), swine erysipelas and glanders. As might be expected, the louse-borne diseases receive due attention. Under the chapter on epidemic typhus the translator, in a footnote, has seen fit to mention prophylactic vaccine and DDT in the control of that disease. Effective use of these two powerful weapons has not yet been generally adopted in the Balkan Countries. The original work stresses the use of convalescent serum both therapeutically and prophylactically and reminds the reader that killed vaccine cannot be prepared in adequate quantities for practical purposes.

While I am not surprised that streptomycin has not reached Eastern Europe I wonder why, by use of footnotes, the Spanish translator has not seen fit to mention this antibiotic in the treatment of some of the bacillary diseases.

The manual contains nothing new and makes no contribution other than being an informative handbook. While student nurses might benefit from an English translation of such an outline, I should not recommend it for the medical profession.

Cincinnati, Ohio

WILLIAM M. GERMAIN

Textbook of Public Health. Ed. 12. By W. M. FRAZER, O.B.E., M.D., Ch.B., M.Sc., D.P.H., Barrister-at-law, Gray's Inn, Medical Officer of Health, City and Port of Liverpool, and Medical Officer to the Liverpool Education Committee; Prof. of Hygiene,

University of Liverpool, and C. O. STALLYBRASS, M.D. (State Medicine), Ch.B., D.P.H., M.R.C.S., L.R.C.P., Order of St. Sava, Deputy Medical Officer of Health, City and Port of Liverpool; and Lecturer in Vital Statistics and Epidemiology, Liverpool School of Tropical Medicine. 571 pp., 78 figs., 42 tables. \$6.50. Baltimore: The Williams & Wilkins Company, 1948.

This is a revision of a well known text covering public health largely as practiced in England. The original text carried quite extensive material on environmental sanitation, vital statistics, epidemiology, infectious diseases, maternity and child welfare, school health, mental hygiene and certain other fields sometimes included in the scope of public health. There has been little change from the previous edition in this material. The principal changes are in the introduction as it deals with the new legal aspects of public health and medical care as they will be affected by the new National Health Service Act.

The authors have prepared, it would seem, an excellent text for medical students studying in Britain. It will also be of interest to others wishing to familiarize themselves with British practice as it pertains to public health.

Detroit

BRUCE H. DOUGLAS

Biological Standardisation of the Vitamins. Ed. 2. By KATHERINE H. COWARD, D. Sc., Reader in Biochemistry, University of London; Head of the Nutrition Department, Pharmaceutical Society of Great Britain. 224 pp., 38 figs., 21 tables. \$5.00. Baltimore, The Williams & Wilkins Company, 1947.

While chemical and microbiologic methods for the determination of a number of the vitamins have largely replaced the older classic biologic methods, certain of the vitamins, notably vitamin D, must still be determined biologically and some authorities feel that a periodic checking of chemical and microbiologic methods by the biologic procedure in the case of several of the other vitamins is desirable. The book under consideration has been a valuable and authoritative reference book on the biologic standardization of the vitamins for the past eleven years. As in the first edition, the first part of the new (second) edition considers in some detail the biologic estimation of vitamins A, B₁, C, D, and E, and the question of the interdependence of the vitamins. This portion of the book is comprehensive and accurate. The second part contains an excellent discussion of mathematical treatment of data and, in the author's words, an elementary introduction to the study of statistical methods applied to vitamin determinations. The book concludes with a careful discussion of means of improving biologic assays with many helpful suggestions based on the author's long experience in this field.

The book is obviously intended for those workers who are engaged in the determination of the vitamin potency of foods and of special preparations for therapeutic use, and in research projects in the vitamin field. For these purposes the book is a real necessity and can be highly recommended.

Detroit

JAMES M. ORTEN

Bioluminescence. By E. NEWTON HARVEY, RUBERT S. ANDERSON, JOHN B. BUCK, AURIN M. CHASE, HENRY EYRING, and FRANK H. JOHNSON as presented at a conference on Bioluminescence, the New York Academy of Sciences. 156 pp., 9 illus. \$2.50. New York: New York Academy of Sciences, 1948.

The phenomenon of bioluminescence has long concerned students of nearly every branch of science as well as every thoughtful person who reflects on the mysteries of nature of which this is one of the most intriguing. Recorded scientific observations indicate that such ancient philosophers as Aristotle and Pliny were interested in this phenomenon, and it is surprising indeed to learn that, as early as 1796, Spallanzani demonstrated that luminescence in the glowworm is dependent upon the presence of water and of oxygen.

The present monograph is in keeping with the high standards of excellence usually found in publications of the New York Academy of Sciences. It includes stimulating discussions by several outstanding authorities on the following topics: a general survey of the field of

bioluminescence; chemical luminescence; the chemistry of *Cyridina luciferin*; the effect of various agents on bacterial luminescence; and a comprehensive discussion of the anatomy and physiology of the light organ in fire-flies—the most widely studied of all luminescent species.

This monograph is heartily recommended not only for scientists investigating this specialized problem, but also for the busy clinical worker who is interested in, and wishes authoritative information on, one of the wonders of nature, bioluminescence. This excellent publication will provide him with several hours of profitable and fascinating reading material.

JAMES M. ORTEN

Chromatography. By HAROLD G. CASSIDY, NORMAN APPLEZWEIG, STIG CLAESSON, VICTOR R. DEITZ, BEVERIDGE J. MAIR, A. J. P. MARTIN, STANFORD MOORE, ROBERT L. PECK, W. A. SCHROEDER, LEO SHEDLOVSKY, WILLIAM H. STEIN, HENRY C. THOMAS, and L. ZECHMEISTER. Editor, Roy Waldo Miner; Consulting Editor, Harold G. Cassidy; Associate Editor, Lothar Salin. pp. 141-326. Volume XLIX, Art. 2. New York: Annals of the New York Academy of Sciences, 1948.

This monograph is composed of a series of papers given at a Conference on Chromatography held by the Section of Physics and Chemistry of the New York Academy of Sciences. The papers are written by recognized authorities in the field and cover the following general topics: history, scope and methods of chromatography, theoretical considerations, partition chromatography and the application of chromatography to the separation of specific substances such as hydrocarbons, stereoisomers, amino acids and streptomycin.

The book is most certainly essential to the library of investigators employing any form of chromatographic analysis. The chapter on the separation and determination of amino acids will undoubtedly prove the most valuable and interesting to clinical laboratory workers.

JAMES M. ORTEN

The Neocortex of Macaca mulatta. Illinois Monographs in the Medical Sciences, Vol. V, No. 4. By GERHARDT VON BONIN and PERCIVAL BAILEY. 163 pp., 40 figs., 62 plates. Urbana, Illinois: University of Illinois Press, 1947.

This book offers much more than its title indicates. It contains chapters on general considerations, fissural patterns, architectural types (heterotypical cortex, homotypical cortex, and allocortex), survey of serial sections, the brain map and interrelations of areas. There are 40 figures and 62 plates and a colored frontispiece of the cortical areas of *Macaca* to illustrate the text. Economo's system of symbols is used. There are also numerous comparisons between the various architectural types of neocortex in man. A detailed study of this work is recommended to neuro-anatomists, neurophysiologists, neuropathologists, neurologists, and neurosurgeons as well as to everyone interested in the morphology of the brain.

Detroit

GABRIEL STEINER

Modern Clinical Psychiatry. By ARTHUR P. NOYES, M.D. (Revised Third Edition). 525 pp. Philadelphia: W. B. Saunders Company, 1948.

This popular text on clinical psychiatry, first published in 1934, appeared in second edition in 1939. The present edition, 36 pages shorter, has been carefully revised and contains three new chapters on Psychotherapy, Shock and Other Physical Therapies, and Child Psychiatry. The format has been improved by an attractive binding and the text made more readable by frequent paragraph headings.

There is additional material on the Rorschach and the Murray Thematic Apperception tests, but the Bender-Gestalt test is not mentioned. The section on electro-encephalography is enlarged, reflecting the increased importance of this procedure. The use of dilantin and tridione in the epilepsies is described. The chapter on involutional melancholia stresses the importance of psychologic and minimizes the importance of physiologic

factors. The author states, "No significant alteration in excretion of ovarian hormone has been established."

An added section on war neurosis emphasizes the use of narcosynthesis and group psychotherapy. An enlarged section on psychosomatic medicine reflects the increased interest in this area during the past decade and briefly summarizes the more important advances in this field. The material on shock therapy is organized in a separate chapter and adequately presented, although it does not include material indicating that depletion of the potassium ion may be an important factor in "the irreversible or prolonged comas".

The added chapter on Child Psychiatry emphasizes the need for greater attention to the developmental factors in mental illness and indicates how clinical psychiatry is shifting its focus from exclusive preoccupation with the more rigid aspects of established mental illness to the more fluid early stages of emotional conflicts and their preventive aspects.

The bibliographies at the end of each chapter have been revised and will be helpful to those who desire additional material. The index is adequate.

Eloise, Michigan

LOUIS S. LIPSCHUTZ

A History of the Heart and the Circulation. By FREDERICK A. WILLIUS, M. D., M. S. in Med., Senior Consultant in Cardiology, Mayo Clinic, Professor of Medicine, Graduate School, University of Minnesota; and THOMAS J. DRY, M. A., M. B., Ch. B., M. S. in Med., Consultant, Section on Cardiology, Mayo Clinic; Associate Professor of Medicine, Graduate School, University of Minnesota. 456 pp., illustrated. \$8.00. Philadelphia and London: W. B. Saunders Company, 1948.

This attractive monograph contains portraits of over 160 contributors to the knowledge of the heart and circulation. It is divided into three sections. The first is a chronologic presentation of knowledge of the heart and circulation from 3,000 B.C. to 1925 A.D., extending from the ancient Egyptian era to the end of the first quarter of the 20th century. The second section contains 20 special biographies, including those of Francis Henry Williams, Rudolph Matas, Sir James Mackenzie, Sir William Osler, and Sir Thomas Lewis. The third section is a chronologic presentation, with reference to the heart and circulation, of historical data according to subjects. These subjects include anatomy, aneurysm, arrhythmia, diagnostic signs, congenital malformations, coronary vessels and their diseases, electrocardiography, milestones in medical education, pathology, physiology and surgery, symptomatology, and treatment.

The authors recognize that since all historical documentary sources were not available to them, some errors might be present in this book. They consider the interpretation of medical data, even those of remote eras, to be a function of the physician rather than of the historian. They have included data from ancient eras which they consider important in the evolutionary development of present day concepts. It is obvious that their claim is justified that the chronologic treatment is preferable to a narrative discussion since it lends clarity and orderliness to their presentation.

The last items mentioned concern several publications in 1925, which include Pardee's observation on the coronary T wave and Warthin's demonstration of spirochetes in the heart in syphilitic myocarditis.

This volume is attractive and carefully prepared. It contains a great deal of information for all those who are interested in diseases of the heart.

Eloise, Mich.

S. E. GOULD

NEWS AND NOTICES

MARYLAND SOCIETY OF PATHOLOGISTS, INC.

The first meeting of the Maryland Society of Pathologists, Inc., was held in Cumberland, Maryland on May 22, 1948. The following officers were elected at the business meeting: Dr. Vernon Norward, President; Dr. Benedict Skitarelle, Vice-President; Dr. H. L. Wollenweber, Secretary-Treasurer; Dr. C. Gardner Warner and Dr. Harold Stewart, Councilors; Dr. C. Wilbur Stewart, Counselor to the American Society of Clinical Pathologists. A constitution was adopted and it was decided to incorporate the Society. A scientific program followed the business meeting.

PENNSYLVANIA ASSOCIATION OF CLINICAL PATHOLOGISTS

The Pennsylvania Association of Clinical Pathologists held its Fall Meeting at the Bellevue-Stratford, Philadelphia, Pennsylvania, October 2-3. Papers presented before the Scientific Session included the following:

The Clinical Pathologist and the Public Health, Dr. A. Parker Hitchens, Philadelphia.
Carcinoma in the Fetus, Dr. Edwin E. Ziegler, Lancaster.

A Survey of the Accuracy of Bacteriological Examinations in Clinical Laboratories, Dr. George R. Lacy, Pittsburgh.

Bone Marrow Biopsy, Dr. Leoncio M. Tocantins, Philadelphia. There was also a Round Table Discussion on the Training of Residents, with Dr. Samuel R. Haythorn, Pittsburgh, acting as moderator.

The officers of the Association are Dr. Frederiek O. Zillesen, Easton, President, Dr. Theodore R. Helmbold, Pittsburgh, Vice-President, and Dr. Henry F. Hunt, Danville, Secretary-Treasurer.

THE AMERICAN SOCIETY FOR THE STUDY OF ARTERIOSCLEROSIS

The next annual meeting will be held on October 31 and November 1, 1948 at the Hotel Knickerbocker in Chicago, Illinois. The officers of the Society are: President, Dr. William B. Kountz, St. Louis, Mo.; Vice-President, Dr. Irvine H. Page, Cleveland, Ohio; Secretary, Dr. O. J. Pollak, Quincy City Hospital, Quincy 69, Mass.

NATIONAL RESEARCH COUNCIL APPOINTS SUBCOMMITTEE ON ONCOLOGY

The Committee on Pathology of the National Research Council has appointed the following Subcommittee on Oncology: Dr. Shields Warren, Chairman, Dr. Balduin Lucké, Dr. Fred W. Stewart, Dr. Harold L. Stewart, Dr. Arthur P. Stout, Dr. Milton C. Winternitz, and Dr. Howard T. Karsner, Chairman of Committee on Pathology, ex officio.

Brig. Gen. Raymond C. Dart, Director of the Army Institute of Pathology, is cooperating with the Committee and the Army Institute of Pathology is making its facilities and resources available and is providing office space for the permanent secretary.

The objectives of the Subcommittee are:

1. Improvement in the teaching of oncology;
2. Dissemination of information on oncology to clinical pathologists, students and teachers of oncology;
3. Establishment of criteria for diagnosis of tumors;
4. Simplification of terminology by recommending a single term for each tumor and listing separately the appropriate synonyms.

The Subcommittee expects to work with existing agencies to promote clarity and unity in tumor nomenclature and classification.

The Executive Secretary of the Subcommittee on Oncology is Dr. I. H. Perry, Army Institute of Pathology, Washington 25, D. C.

PERSONALS

Dr. Cecil A. Krakower has been promoted to the rank of Professor of Pathology at the

University of Illinois College of Medicine. He also will serve as Associate Pathologist in the University's 428-bed Research and Educational Hospitals.

Dr. F. William Sunderman, Professor of Clinical Pathology and Director of Laboratory of Clinical Medicine at Temple University Medical School, has been appointed head of the Department of Clinical Pathology at the Cleveland Clinic Foundation.

PRACTICE OF MEDICINE BY A HOSPITAL

The following opinion (No. 48-32) is reprinted from the "Opinions of the Attorney General of California", Volume 11, June 7, 1948 #10.

Subject: MEDICAL PRACTICE: Corporation Operating Hospital Cannot Pay a Physician a Fixed Salary and Bill Patients for Services Rendered by the Physician at Rates Unrelated to His Salary, Since This Would Constitute Illegal Practice of Medicine by a Corporation.

Requested by: BOARD OF MEDICAL EXAMINERS.

Opinion by: FRED N. HOWSER, Attorney General, and E. G. FUNKE, Deputy.

The Board of Medical Examiners has submitted the following questions:

1. Is a corporation or an association of laymen operating a private, nonprofit hospital permitted to practice any system or mode of treating the sick or afflicted in this State?
2. If a corporation operating a private, nonprofit hospital enters into a contract with a physician under which the physician will perform professional services in the hospital and receive a fixed salary, and the corporation will thereupon bill the patient for the professional services rendered by the physician at rates that have no bearing on the physician's salary, is the corporation violating any of the provisions of the Medical Practice Act?

The conclusions reached are summarized as follows:

1. No one is permitted to practice any system or mode of treating the sick or afflicted in this State unless he is licensed in accordance with the provisions of section 2000 *et seq.*, Business and Professions Code. Corporations or other artificial legal entities are specifically mentioned in section 2008 as having no professional rights, privileges or powers, and may, therefore, not be licensed to so practice.
2. The employment of a licensed physician by a corporation and the subsequent billing of the patients by the corporation, as referred to in the second question, would constitute illegal practice of a system or mode of treating the sick or afflicted in this State and is, therefore, prohibited by law.

ANALYSIS

The Board of Medical Examiners advises that a private, nonprofit hospital, operating in the State and owned by a corporation, contemplates entering into a contract with a duly licensed physician and surgeon who specializes as a pathologist. They propose that the physician and surgeon will perform professional services for hospital patients and receive, therefore, a fixed salary. The corporate owner of the hospital proposes to bill separately each private patient for the professional services that have been rendered to such private patient by the pathologist. They propose that such charges are to be independent of the ordinary regular charge for hospital bed, board and usual hospital services and further, that the rate of charge will have no bearing on the salary that the pathologist will receive from the corporation.

The courts have made it abundantly clear, as is hereafter shown, that corporations are prohibited from engaging in the practice of any system or mode of treating the sick or afflicted in this State. The pronouncements of the courts also, in our opinion, require the conclusion that the arrangement contemplated by the hospital in question falls within the same prohibition.

The California Legislature has enacted a Corporations Code. In the Corporations Code there are found various provisions governing the formation, powers and duties of corporations as a whole. Since a corporation is a "Creature Created by Statute", it has only such

powers as the statutes give to it. Nowhere in the Corporations Code is a corporation given specific authority to practice the healing arts.

We must, of course, call attention to the fact that certain nonprofit corporations may be formed for the purpose of defraying or assuming the cost of professional services of licentiates of the healing arts. Section 9201, Corporations Code, formerly Section 593 (A) Civil Code, so provides. However, the same section except as expressly permitted therein does not authorize the formation of any corporation for the purpose of rendering the professional services regulated by Division 2 of the Business and Professions Code. Likewise, Chapter 1 of Division 2 of the Health and Safety Code, governing the operation of clinics and hospitals, specifically provides (Section 1214) that the provisions of said chapter do not authorize any person other than a licentiate of a healing art to engage directly or indirectly in the practice of medicine.

The opinion of the Supreme Court in *California Physicians' Service v. Garrison*, 28 Cal. 2d 790, construed the provisions of Corporations Code 9201, formerly Civil Code 593 (A), and particularly the authorizing of the incorporation of a physician's service. The court therein states (page 802): "The Legislature by enacting section 593 (A) of the Civil Code, expressly authorized the organization of corporations such as California Physicians' Service. By this enactment the State's social policy in regard to the corporate practice of medicine, to the limited extent specified, has been determined and the courts are bound thereby." (Emphasis added.)

Further, a corporation, although considered by law as a legal entity, and to have in many respects all the rights and privileges of an individual person, nevertheless is physically unable to fulfill the educational requirements or to take the examination required of all persons who seek to secure a license to practice the healing arts. Thus, even though section 2008, Business and Professions Code, did not specifically state that a corporation has no professional standing, nevertheless it would be a physical impossibility for a corporation to be a licentiate of a healing art.

Our courts have on numerous occasions held that a corporation may not engage in the practice of medicine. The opinion of the Supreme Court in *Pacific Employers Insurance Co. v. Carpenter*, 10 Cal. App. 2d. 592, 594, contains a comprehensive discussion which is pertinent. The following quotation summarizes the courts' views on this subject:

"It is well settled that neither a corporation nor any other unlicensed person or entity may engage, directly or indirectly, in the practice of certain learned professions including the legal, medical and dental professions. (Cases cited.) Under the foregoing authorities it is clearly declared unlawful for a corporation to indirectly practice any of said professions for profit by engaging professional men to perform professional services for those with whom the corporation contracts to furnish such services. In other words, said authorities declare that said professions are not open to commercial exploitation as it is said to be against public policy to permit a 'middleman' to intervene for profit in establishing the professional relationships between the members of said professions and the members of the public". (Emphasis added.)

In *People v. Pacific Health Corporation*, 12 Cal. 2d 156, 158, the court stated that: "It is an established doctrine that a corporation may not engage in the practice of such professions as law, medicine or dentistry (citing cases)." The appellant, Pacific Health Corporation, contended, however, that it did not itself undertake to perform medical services, but merely to furnish competent physicians; that the physicians and surgeons were not to be employed by it on a salary basis, nor directed by it, but were to be compensated for actual professional services after they were rendered; and the corporation's theory was that the doctors, under its arrangement, were to be independent contractors and that, therefore, the corporation would be absolved of the charge of practicing medicine. The court said:

"We are unable to agree that the policy of the law may be circumvented by technical distinctions in the manner in which the doctors are engaged, designated or compensated by the corporation. The evils of divided loyalty and impaired confidence would seem to be equally present whether the doctor received benefits from the corporation in the form of salary or fees. And freedom of choice is destroyed, and the elements of solicitation of

medical business and lay control of the profession are present whenever the corporation seeks such business from the general public and turns it over to a special group of doctors."

This argument that the mere ownership of a hospital where medical services are rendered by the owner's licensed employees does not in itself constitute the practice of medicine (*i.e.*, that the practice of medicine involves actual treatment of persons), was also rejected by our courts when applied to the practice of dentistry. See *Painless Parker v. Board of Dental Examiners*, 216 Cal., 285, 296. Appellant in that case contended that there was a distinction between the practice of dentistry which the statutes undertook to regulate, and the purely business side of the practice and that the management and conduct of the business side by a layman was not prohibited by the statute, and that such attempted prohibition would be unconstitutional. We refer to the well considered opinion of the court, wherein are given the reasons for the rejection of this contention made by appellant.

It may be contended that the pathologists in the situation presented would merely examine and diagnose an illness and, therefore would not be practicing medicine. But our courts have held that diagnosis is as much a part of the practice of medicine as is the administration of remedies. In fact, section 2141, Business and Professions Code, declares that one who diagnoses any illness is engaging in the practice of medicine (see *People v. Jordan*, 172 Cal. 391).

Throughout the opinions cited one will note that the courts have indicated that the practice of medicine by corporations for profit, through the employment of licensed physicians, has a tendency to debase the profession, is not in the interests of the safety, health and welfare of the public, and therefore is contrary to public policy. The right to practice medicine and surgery under a license by the State is a personal privilege. It cannot be delegated. Therefore, a corporation or other unlicensed person may not engage in the practice of medicine by employing one who is licensed to do the things which constitute the practice of the profession. Were the rules otherwise, one would find a licensed physician accepting directions and instructions in the diagnosing and treating of ailments from a corporation or from an individual who is not a licensed practitioner.

This opinion was sent as a memorandum to voluntary hospitals and institutional members of California Hospitals from Thomas F. Clark, Executive Secretary of the Hospital Association, dated June 22, 1948.

VENEREAL DISEASE RECORDS OF VETERANS AVAILABLE

The Veterans Administration announces that it has in its custody the majority of syphilis records of those Army personnel who were treated for this disease while in active service, and in many instances can procure informative data from the syphilis records of other than Army personnel. It is thought that many physicians treating veterans for syphilis as private patients would find a resumé of the syphilis record useful since the details of treatment, results of spinal fluid examinations and blood serologies are incorporated in the records. Resumés of these records are available to physicians who are treating such veterans, provided authorization for the release of the data is given by the veteran. Requests for the resumés accompanied by an authorization for the release of the information, dated and signed by the veteran, should be addressed to the Dermatology and Syphilology Section, Veterans Administration, Munitions Building, Washington 25, D. C. It is most important that the veteran's Service Serial Number and other identifying information such as the date of enlistment, the date of discharge, rank and organization be included. Ordinarily, the resumés can be furnished in approximately two weeks from the date of the receipt of the request and signed authorization.

THE VITAMIN K TOLERANCE TEST*

PAUL N. UNGER, M.D.,† MURRAY WEINER, M.D., AND
SHEPARD SHAPIRO, M.D.

From the Third (New York University) Division, Goldwater Memorial Hospital, Welfare Island, and the Department of Medicine, New York University, College of Medicine, New York, New York

The present paper is a continuation of our previous studies in which we demonstrated that the prothrombin time following large doses of vitamin K may be utilized as a liver function test of high sensitivity.¹⁷ The investigation has also been extended to include some determinations of concurrent variations in the fibrinogen concentration of the plasma.

PROCEDURE

In the first investigation only the alteration in prothrombin level or activity was studied. The method used for estimation of the prothrombin time has been described previously.¹⁶ It includes estimation of the prothrombin time of whole and diluted (12.5 per cent) plasma. Inasmuch as clotting of whole plasma occurs so rapidly that fine changes may be missed, significant variations may be detectable in the prothrombin time by the use of diluted (12.5 per cent) plasma.^{4, 9}

The resting level of prothrombin is established by successive estimations of the diluted (12.5 per cent) plasma prothrombin time made on several consecutive days. Large doses of vitamin K are given intravenously and prothrombin estimations made on each of several days following the injections. The normal plasma prothrombin time of diluted (12.5 per cent) plasma ranges from 37.0 to 42.0 seconds.

Synthetic vitamin K (Synkayvite "Roche", tetra-sodium-2-methyl-1,4-naphthohydroquinone diphosphoric acid ester) is given intravenously in daily doses of 76 mg. each (equivalent to 60 mg. Hykinone, Abbott, 2-methyl-1,4-naphthoquinone sodium bisulfate), for four successive days after the resting level is established. The prothrombin time is estimated daily prior to each injection and for one day following the last injection. Estimations are made in duplicate and on freshly drawn specimens of blood. Lipemic bloods may interfere with the accuracy of the estimations.¹² Thromboplastin is prepared daily. The preparation used was one of standardized potency made from fresh rabbit lung desiccated *in vacuo*. Immediate estimation of the prothrombin time is preferable, but if this is not possible, the plasma may be stored at ice box temperature for no longer than four hours. The blood should be allowed to attain room temperature gradually after its removal from the refrigerator.

The fibrinogen concentration was determined by establishing the protein content of the plasma before and after the contained fibrinogen was precipitated

* Read by title at the Twenty-Sixth Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October, 1947. Received for publication, May 6, 1948.

† Present address: Miami Beach, Florida.

and removed. This method has an error of 10 to 15 per cent. The normal range is from 250 to 550 mg. per 100 ml. The cephalin flocculation tests were performed using Difeo cephalin, the readings being made at the end of twenty-four and forty-eight hours. The bromsulfalein retention was determined by giving 5 mg. of the dye per Kg. body weight and taking blood samples thirty minutes after injection. Retention of more than 10 per cent bromsulfalein at the end of thirty minutes was considered abnormal.

The test of prothrombin response to large doses of vitamin K is hereafter referred to as the vitamin K tolerance test.¹⁷ The results obtained with this procedure were correlated, when feasible, with results of the following tests: bromsulfalein, icterus index, van den Bergh, cephalin flocculation, total cholesterol and cholesterol esters (by Bloor's technique), total protein content of the blood and albumin-globulin ratio.

RESULTS

One hundred and thirty-two vitamin K tolerance tests were made on 123 persons. This represents a complete resumé of all our data obtained up to the present time. In 9 of the patients, fibrinogen determinations were made concurrently with the prothrombin estimations.

The results of the vitamin K tolerance test were interpreted as negative, borderline, or positive depending on the magnitude of the alteration in the prothrombin time. A negative test (Fig. 1) is one in which the change conforms to one of the following patterns after the injection of large doses of vitamin K: (a) The resting level is normal and the serial estimations of prothrombin time continue within the normal range; (b) the resting level is prolonged (hypoprothrombinemia) and the prothrombin time returns to normal after vitamin K is given; (c) the resting level is normal and the prothrombin time is changed to hyperprothrombinemic levels after the vitamin K is administered. The negative series yielded maximal levels below 45.0 seconds.

A positive test (Fig. 2) is one which yields one of the following curves: (a) The resting level is prolonged (hypoprothrombinemia) and the prothrombin time does not return to normal after vitamin K is given (Curves A and B); (b) the resting level is prolonged (hypoprothrombinemia) and the prothrombin time is further increased after vitamin K (Curve C); (c) the resting level is within the normal range; after vitamin K is administered the prothrombin time becomes prolonged to exceed 47 seconds in one or more determinations during the period of test (Curve D).

The establishment of the dividing point at 45 seconds between normal and abnormal responses to the administration of large doses of vitamin K is admittedly an arbitrary limit. A certain degree of flexibility, therefore, should be used in interpreting results a few seconds over 45 until further experience shows whether or not this level is absolutely valid as the point of demarcation. Results between 45 and 47 seconds, therefore, have been classified as doubtful for the present.

Representative examples of the concurrent variation in prothrombin time and

NEGATIVE TESTS

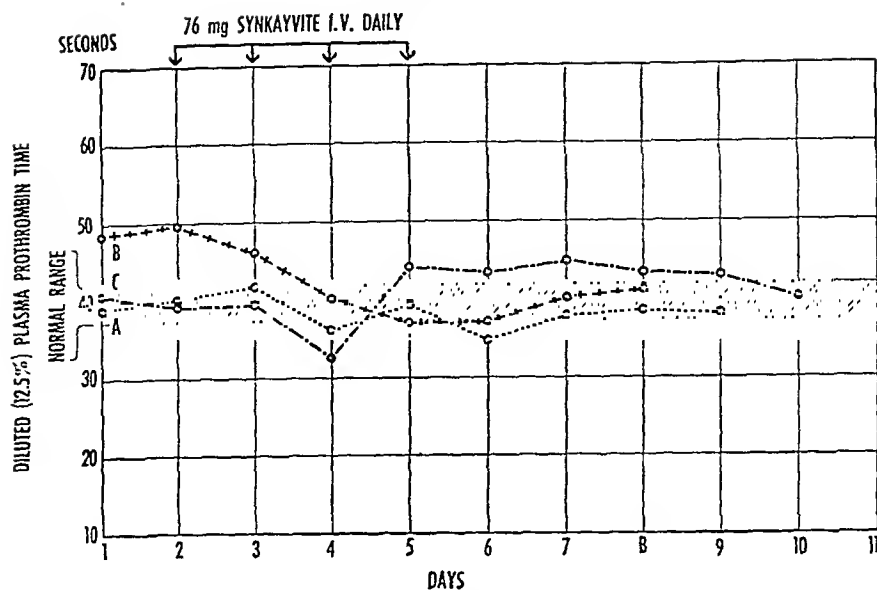


FIG. 1. Curve A remains within the normal range throughout the period of the test. Curve B shows initial hypoprothrombinemia with restoration to normal after administration of vitamin K. This is typical of nutritional vitamin K deficiency. Curve C indicates temporary hyperprothrombinemia after large doses of vitamin K.

POSITIVE TESTS

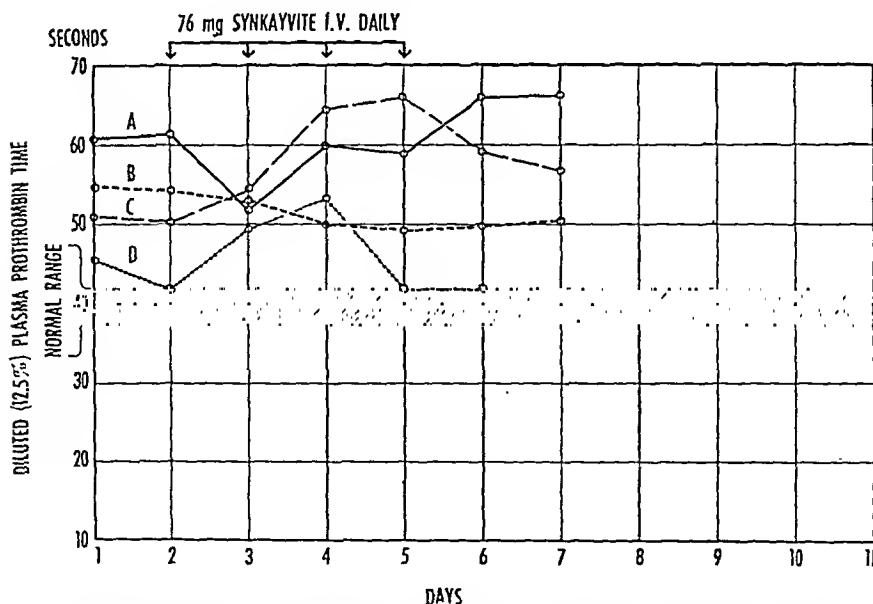


FIG. 2. Curves A and B show initial hypoprothrombinemia with partial restoration toward normal after vitamin K administration followed by further prolongation beyond the resting level. Curve C shows initial hypoprothrombinemia unaltered by vitamin K, and curve D reveals further extension of prothrombin time promptly following vitamin K administration and eventual restoration to normal.

fibrinogen concentration are presented in Figure 3. There occurred no significant change in fibrinogen level to parallel the shift in prothrombin time. The fibrinogen values were never reduced to a point where they might have influenced the prothrombin time. It is noteworthy that in some of the patients with liver disease the fibrinogen values actually increased beyond the resting levels, although the existing hypoprothrombinemia failed to improve.

ANALYSIS OF RESULTS

The cases have been arranged in the tables in progressively increasing order from the viewpoint of the maximum prothrombin time reached during the test. It is apparent from Table 1 that a dividing line may be established in the range between 45 and 47 seconds in results of tests on patients with and on patients without liver disease. Patients 1 through 49 include those in whom the maximum prothrombin time attained during the test was below 45.0 seconds. The data indicate that there were no diseases which are associated with impaired liver function in this group with the exception of two patients (No. 10, 26). There were five instances of hepatomegaly due to congestive failure (No. 8, 25, 39, 47, 48) and two patients with amyloid disease (No. 23, 38). One cachectic patient (No. 21) had a nutritional hypoprothrombinemia which responded slowly to vitamin K therapy. In none of these patients was there reason to expect impaired liver function.

Forty bromsulfalein tests were made in the group of 49 patients who exhibited negative vitamin K tolerance tests. Five of these yielded more than 10 per cent retention of bromsulfalein at the end of thirty minutes. Only one of these 5 patients (No. 10) presented clinical evidences suggestive of impaired liver function. In addition, patient 26, in whom mild cardiac cirrhosis was proved at autopsy, also had a negative bromsulfalein test.

In 40 of the 49 patients cephalin flocculation tests were made. Eighteen yielded results of 2 plus or greater. Only one of the 18 patients showed impaired function with confirmatory clinical or laboratory evidence of liver disease. The remaining 17 were, therefore, considered to be false-positive cephalin flocculation tests.

In the second group (patients No. 50 to 59) maximum prothrombin time attained during the test was in the range of 45 to 47 seconds. These results are for the present considered of doubtful significance. In this group, 8 had clinical manifestations of diseases which may be associated with impaired liver function. The bromsulfalein test was made in 7 of the patients, in 2 of whom there was abnormal retention. Cephalin flocculation was studied in 9 of the patients, in 4 of whom the test was abnormal. It is of particular interest to note the lack of correlation between the results of the cephalin flocculation and bromsulfalein retention tests in this group of patients.

Of the remainder of the series (patients No. 60 through 123), 8 showed maximum prothrombin times ranging from 47.8 to 49.4 seconds inclusive. Seven of these 8 patients had clinical disturbances known to be associated with impaired liver function. The eighth patient had hepatosplenomegaly of cardiac origin.

The bromsulfalein test was made in 7 of the 8 patients; it was abnormal in 3. Cephalin flocculation tests in 7 of the 8 patients yielded 2 positive results.

Patients No. 68 to 107 inclusive comprise a group of 40 patients in whom the maximum prothrombin time reached during the vitamin K tolerance test ranged from 50.0 to 59.0 seconds, inclusive. Twenty-eight of the patients in this group showed definite clinical evidence of liver disease. The remaining 12 showed no signs of specific hepatic disease. It is important to note that 5 of these 12 patients were severely anemic, and one suffered from moderate anemia and nutritional deficiency. Five exhibited no clinical evidence of liver disease but they were over 75 years of age.* The remaining person in this group (No. 89) was originally studied as a control and exhibited an abnormal vitamin K tolerance test on three separate occasions. He gave no history of any type of liver disease, but had recently completed a period of military service in an area where infectious hepatitis was endemic. The liver was palpable two fingerbreadths below the costal margin, and the icterus index was 11.2 units. All other liver function tests were negative.

The last group of 16 patients (No. 108 to 123) includes those in whom the maximum prothrombin time obtained during the vitamin K tolerance test ranged from 60.2 to 155.0 seconds, inclusive. All of them showed unequivocal evidences of liver disease, except for one 78 year old patient with a history of transient icterus.

Table 2 summarizes the results and indicates that the vitamin K tolerance test exhibits excellent correlation with clinical findings and that it may be used as a test of liver function.

FIBRINOGEN CONCENTRATION AND PROTHROMBIN TIME

A study was made to learn whether or not changes occurred in the fibrinogen concentration of the plasma during the vitamin K tolerance test and if they were of sufficient magnitude to influence the prothrombin time.⁶

For this purpose, 9 patients were studied. The fibrinogen content of the plasma was established prior to, during and immediately after the test. Table 3 gives the summary of the clinical and laboratory data in the 9 instances, including 8 with liver disease of varying intensity and one (first case listed) with no demonstrable evidence of liver impairment. Figure 3 illustrates two of these experiments. In 4 patients the changes noted in the fibrinogen values fell well within the experimental range of error for determination of this protein. In 4 others, the fibrinogen values at the termination of the test were in excess of the resting levels. In one instance the fibrinogen content was somewhat lower than the resting level at the completion of the test. It is concluded that the fluctuations

* Rafsky¹⁴ has demonstrated the presence of impaired liver function in the aged. We have been impressed by the frequency with which the very old have impaired liver function tests in the absence of clinical evidence of specific liver disease. Our 49 patients with negative vitamin K tolerance tests include only one patient over 75 years of age. Of the 74 patients with doubtful or positive tests, there are 17 over 75 years old, 11 of whom had no clinical evidence of specific liver disease.

TABLE 1

COMPARISON OF CLINICAL FINDINGS WITH VITAMIN K TOLERANCE TESTS AND OTHER LIVER FUNCTION TESTS

CASE NO.	AGE	DIAGNOSIS	ENLARGED LIVER	CLINICAL LIVER DISEASE	PROTHROMBIN TIME (SEC.) 12.5 PER CENT PLASMA			VITAMIN K TOLERANCE TEST	BROM-SULF-ALEIN RETENTION, %, 30 MIN.	ALB./GLOB., GM. PER 100 ML. BLOOD	CEPHALIN FLOCCULATION	CHOLESTEROL/ESTERS, MG. PER 100 ML. BLOOD
					Resting Before Test	Maximum During Test	Resting After Test					
150		Duodenal ulcer		—	40.8	32.4	35.0	Neg.	0	5.59/1.73	0	164/113
256		Carcinoma pancreas ^a	+	—	31.6	34.8	29.6	Neg.	0	3.9/2.8	2+	320/134
377		Rheumatoid arthritis		+a	34.6	35.0	32.4	Neg.	0		3+	
458		Carcinoma pancreas, icterus		—	>300	36.6	46.2	Neg.		2.4/2.3	0	435/-
550		Hypertension		—	36.4	37.0	31.6	Neg.	2		0	
658		Arteriosclerosis		—	42.0	37.0	36.5	Neg.	4		3+	365/203
747		Rheumatoid arthritis		—	36.2	37.2	36.4	Neg.	0	4.1/2.2	0	
848		Hypertension, polyserositis	+	—	38.0	37.4	32.2	Neg.	2	4.2/4.5	2+	
925		No disease		—	37.0	37.4	33.5	Neg.				
1036		Alcoholic fatty liver	+	+	37.3	37.8	35.0	Neg.	14	3.3/2.7	4+	244/155
1150		Diabetes mellitus		—	35.5	38.0	38.4	Neg.	14		2+	
1229		No disease		—	40.4	38.2	35.0	Neg.				
1338		Multiple sclerosis		—	41.5	38.4		Neg.	0		1+	
1439		Multiple sclerosis		—	38.0	38.6	39.4	Neg.	0		1+	
1540		Multiple sclerosis		—	39.5	38.8	40.2	Neg.	0			
1663		Rheumatoid arthritis		—	43.5	38.8	32.4	Neg.	12	3.4/3.3	2+	256/124
											0	
											1 wk. later	
1754		History hepatitis		—	50.2	38.8	35.4	Neg.	0		0	255/178
1835		Multiple sclerosis		—	46.5	39.6	41.2	Neg.	0	4.6/2.0	3+	
1936		Alcoholic neuritis		—	38.6	39.8	39.9	Neg.	6	3.1/3.4	0	203/174
2043		Chronic pyelonephritis		—	36.5	40.6	41.0	Neg.		3.0/2.7	3+	227/139
2125		Simmond's disease ^a		—	60.8	40.6	50.0	Neg.	2	2.1/5.0	2+	161/83
2229		Multiple sclerosis		—	39.8	40.8	37.6	Neg.			3+	
2342		Amyloidosis	+	—	35.8	41.2	42.0	Neg.	8	3.3/3.4	0	275/-
2429		Transverse myelitis		—	41.7	41.2	38.8	Neg.	0	3.9/2.8	2+	185/141
2559		Heart failure	+	—	52.9	41.3		Neg.	8	4.2/2.4	1+	212/161
2654		Cardiac cirrhosis ^a	+	+	36.9	41.8	41.8	Neg.	0	4.9/2.4	0	258/141
2738		Diabetes mellitus		—	39.5	41.8	37.8	Neg.	0	3.7/2.3	0	244/182
2834		Multiple sclerosis		—	40.4	41.8	38.0	Neg.	0		0	
2970		Coronary sclerosis		—	40.8	41.8	36.0	Neg.	0	2.8/2.8	4+	256/137
3034		Multiple sclerosis		—	42.4	42.0	35.2	Neg.	0	4.5/2.4	2+	

VITAMIN K TOLERANCE TEST

TABLE 1—Continued

CASE NO.	AGE	DIAGNOSIS	ENLARGED LIVER	CLINICAL LIVER DISEASE	PROTHROMBIN TIME (SEC.), 12.5 PER CENT PLASMA			VITAMIN K TOLERANCE TEST	BROM-SULF-ALEIN RETENTION, %, 30 MIN.	ALB./GLOB., GM. PER 100 ML. BLOOD	CEPH-ALIN FLOCCULATION	CHOLESTEROL/ESTERS, MG. PER 100 ML. BLOOD
					Resting Before Test	Maximum During Test	Resting After Test					
3132		No disease	—	—	41.8	42.0	42.8	Neg.	0	4.3/2.4	4+	
3226		Friedreich's ataxia	—	—	40.8	42.2	36.8	Neg.	10		0	250/91
3334		Syringomyelia	—	—	43.7	42.4	37.2	Neg.	2	3.1/2.1	0	
3455		Macrocytic hyperchromic anemia	—	—	37.2	42.4	42.8	Neg.			1+	500/431
3537		Friedreich's ataxia	—	—	45.7	42.5	40.8	Neg.	0	3.2/2.3	2+	
3655		Nephrotic syndrome(?) ^b	—	—	39.0	42.6	41.6	Neg.	0		0	178/97
3742		Hypertension	—	—	49.6	42.6		Neg.	5	3.0/3.1	0	358/170
3849		Amyloidosis ^b	+	+	34.0	43.4	36.5	Neg.	1	1.9/2.9	0	466/221
3965		Cardiac failure	+	+	40.0	43.8	34.0	Neg.	14	3.9/2.8	0	
4031		No disease	—	—	38.6	43.8	43.0	Neg.				
4137		Rheumatoid arthritis	—	—	38.0	43.8	37.0	Neg.	7	3.9/2.6		
4225		No disease	—	—	40.5	44.2	44.6	Neg.	0		0	310/184
4342		Parkinsonism	—	—	43.1	44.2	42.6	Neg.	8	5.0/2.6	2+	161/94
4468		Heart failure	—	—	40.8	44.2	35.0	Neg.	26	3.0/3.5		
4523		Gonorrheal arthritis	—	—	38.7	44.2	31.8	Neg.			3+	
4643		Friedreich's ataxia	—	—	36.8	44.3	35.0	Neg.	0	3.5/2.5	1+	188/110
4763		Heart failure	+	+	38.9	44.4	46.2	Neg.	2	3.9/3.0	1+	245/173
4843		Heart failure	+	+	45.0	44.4	41.8	Neg.	0		0	
4940		Multiple sclerosis	—	—	40.1	44.8	42.8	Neg.	0	3.0/3.9	3+	171/109
5060		Arteriosclerosis	—	—	38.3	45.0		?	7	3.9/2.5	0	178/102
5178		Recurrent cholangitis ^{b, c}	+	+	39.4	45.2	44.0	?				
5261		History hepatitis 18 months ago	+	+	37.3	45.6	40.5	?	2	2.6/3.6	0	148/75
5337		Alcoholic, fatty liver, icterus ^b	+	+	42.4	46.2	39.5	?		3.9/2.2	0	278/215
5474		Heart failure	+	+	41.0	46.2	42.4	?	?	2.8/2.7	3+	217/116
5577		Hereditary telangiectasis, anemia	+	+	41.0	46.4	39.8	?	?	2.6/2.6	3+	
5620		Amyloidosis	+	—	39.0	46.8	39.5	?	6	4.7/2.6		120/75
5758		Laennee's cirrhosis	+	+	34.8	46.8	36.8	?	32	3.3/2.4	0	235/127
5864		Obstructive biliary cirrhosis ^b	+	+	57.4	46.8	46.4	?		3.1/3.0	4+	182/62
5959		Diabetes, fatty liver	+	+	39.0	47.0	43.4	?	18	2.9/3.1	0	248/131
6049		Fatty cirrhosis	+	+	48.6	47.8	47.8	Pos.	20	4.75/3.24	0	143/76
6168		Heart failure, anasarca ^a	+	+	53.9	48.2	48.0	Pos.	40	2.8/2.0	2+	227/125

TABLE 1—Continued

CASE NO.	AGE	DIAGNOSIS	ENLARGED LIVER	CLINICAL LIVER DISEASE	PROTHROMBIN TIME (SEC.), 12.5 PER CENT PLASMA			VITAMIN K TOLERANCE TEST	BROM-SULF-ALEIN RETENTION, % 30 MIN.	ALB./GLOB., GM. PER 100 ML. BLOOD	CEPHALIN FLOCCULATION	CHOLESTEROL/ESTERS, MG. PER 100 ML. BLOOD
					Resting Before Test	Maximum During Test	Resting After Test					
62	66	Heart failure	+	—	38.1	48.8	42.8	Pos.		2.9/3.5	0	258/137
63	79	Pernicious anemia		+a	38.7	48.8	41.3	Pos.	0		0	238/132
64	67	Laennec's cirrhosis	+	+	44.1	48.8	41.0	Pos.	10	3.0/3.2	3+	158/77
65	75	Hypertension		+a	45.2	49.2	38.8	Pos.	25	3.8/2.2		
66	38	Laennec's cirrhosis	+	+	44.8	49.2	40.5	Pos.	2	3.7/2.3	0	279/147
67	74	Recurrent cholangitis, ascites	+	+	48.6	49.4	48.4	Pos.	0	4.1/2.1	0	147/108
68	36	Rheumatoid arthritis, gold toxicity		—	39.6	50.0	42.8	Pos.	0		2+	265/180
69	32	Infectious hepatitis, 6 mo. ago		+	40.5	50.1	48.4	Pos.				
70	23	Infectious mononucleosis 6 mo. ago	+	+	42.3	50.1	50.1	Pos.				
71	60	Carcinomatosis liver, ascites ^a	+	+	36.9	50.2		Pos.	32	3.0/2.0		167/84
72	40	Osteosclerotic anemia	+	+a	37.6	50.3	44.3	Pos.				
73	60	Heart failure ^a	+	+	49.8	50.8	49.0	Pos.	20	2.9/2.9	3+	198/74
74	83	Heart failure	+	+	56.6	50.8		Pos.		4.3/2.8	0	235/132
75	38	Laennec's cirrhosis	+	+	51.0	50.8	49.0	Pos.	12	3.3/4.1	0	246/132
76	59	Laennec's cirrhosis, erythremia	+	+	49.5	50.8	41.6	Pos.	10	4.4/2.6	0	245/147
77	58	Portal cirrhosis, icterus	+	+	51.8	50.9	36.0	Pos.	45	3.34/2.84	4+	193/119
78	68	Recurrent cholangitis, liver abscess ^a	+	+	62.1	51.2	55.0	Pos.		2.6/2.6	4+	190/114
79	60	Multiple myeloma, anemia		+a	46.1	51.4	47.2	Pos.	2	3.1/5.5		125/64
80	82	Pernicious anemia	+	+a	52.0	51.4		Pos.	18	3.2/2.0		143/102
81	91	Ochronosis		+a	52.0	51.4	52.0	Pos.	0	3.5/3.2	0	273/161
82	80	Osteoarthritis		+a	55.4	51.4		Pos.	16	3.5/2.2	0	115/89
83	79	Pernicious anemia		+a	46.5	51.6	45.6	Pos.		2.4/2.3		
84	76	Recurrent cholangitis		+	41.1	51.8	43.0	Pos.	10	4.1/2.4	0	203/144
85	40	Heart failure	+	+	46.1	51.8		Pos.	4	4.2/3.8	2+	196/125
86	40	Toxic hepatitis	+	+	46.9	52.8	50.8	Pos.	5	3.7/5.4	4+	212/138
87	75	Coarctation of aorta ^a		+a	56.4	53.0	52.6	Pos.	32	3.4/3.2	2+	102/51

TABLE 1—Continued

CASE NO.	AGE	DIAGNOSIS	ENLARGED LIVER	CLINICAL LIVER DISEASE	PROTHROMBIN TIME (SEC.), 12.5 PER CENT PLASMA			VITAMIN K TOLERANCE TEST	BROM-SULF-ALEIN RETENTION, %, 30 MIN.	ALB./GLOB., GM. PER 100 ML. BLOOD	CEPHALIN FLOCCULATION	CHOLESTEROL/ESTERS, MG. PER 100 ML. BLOOD
					Resting Before Test	Maximum During Test	Resting After Test					
88	27	Acute hepatitis 15 mo. ago		+	43.0	53.0	48.8	Pos.		4.6/2.1	2+	244/198
89	28	No disease	+	+	42.0	53.2	48.2	Pos.	0	4.4/2.1	0	270/187
90	38	Laennec's cirrhosis, ascites ^b	+	+	48.6	53.2	45.8	Pos.		3.8/2.2	1+	190/163
91	42	Laennec's cirrhosis, anasarca ^b	+	+	54.0	53.2	42.0	Pos.	2	2.2/2.7	0	208/98
92	61	Recurrent cholecystitis	+	+	48.7	53.2	50.6	Pos.	12	4.7/2.6	2+	300/217
93	50	Laennec's cirrhosis, icterus	+	+	51.0	53.4	45.2	Pos.	12	2.7/3.5	3+	127/57
94	60	Multiple myeloma, anemia		+a	44.7	53.8	45.0	Pos.	0	2.5/4.5	0	124/52
95	76	Recurrent cholangitis ^b	+	+	39.0	53.8	52.4	Pos.	15	3.0/3.0	3+	200/111
96	38	Laennec's cirrhosis	+	+	45.0	54.0	49.8	Pos.		3.3/3.6	0	312/168
97	56	Chronic alcoholism		+	38.3	54.0	46.2	Pos.	6	3.5/3.9	2+	225/110
98	85	Arteriosclerosis		+a	48.4	54.2	47.0	Pos.	45	4.16/2.86	1+	185/123
99	62	Heart failure	+	+	56.0	54.4	53.1	Pos.	34	3.4/3.2	2+	181/91
100	44	Heart failure	+	+	47.2	55.0	41.4	Pos.		3.6/2.8	0	165/94
101	85	Hypertension		+a	52.9	55.4	47.5	Pos.	28	3.7/3.4	0	129/88
102	63	Diabetes mellitus	+	+	54.2	55.7	50.0	Pos.		2.5/2.1	3+	
103	62	Chronic alcoholism	+	+	58.2	56.0	48.6	Pos.	0		1+	360/199
104	35	Heart failure	+	+	50.2	56.2	45.8	Pos.	2	3.1/2.2	0	193/84
105	60	Laennec's cirrhosis		+	71.4	57.8	62.6	Pos.	15	3.0/4.4		146/79
106	47	Heart failure	+	+	45.8	58.2	54.2	Pos.	2	3.4/2.1	2+	222/128
107	71	Banti's syndrome ^b		+	57.1	59.0	59.0	Pos.	24	4.0/2.7	3+	121/83
108	64	Felty's syndrome ^a	+	+	59.0	60.2	52.0	Pos.	0	1.9/2.6	2+	118/58
109	58	Laennec's cirrhosis	+	+	53.6	60.4	60.0	Pos.	15	3.9/2.9	2+	115/59
110	67	Carcinomatosis liver	+	+	50.0	61.3	42.8	Pos.	12	3.5/1.9		157/76
111	82	Laennec's cirrhosis	+	+	50.7	66.4	58.8	Pos.	32	3.8/2.3	4+	159/97
112	61	Laennec's cirrhosis, ascites ^b		+	61.9	67.2	67.7	Pos.	1	1.9/3.3	0	114/57
113	49	Laennec's cirrhosis	+	+	57.3	68.3	60.0	Pos.	36	2.7/2.3	0	
114	54	Chronic toxic hepatitis	+	+	59.6	73.7	50.0	Pos.	10	4.2/3.4		167/110

TABLE 1—*Concluded*

CASE NO.	AGE	DIAGNOSIS	ENLARGED LIVER	CLINICAL LIVER DISEASE	PROTHROMBIN TIME (SEC.), 12.5 PER CENT PLASMA			VITAMIN K TOLERANCE TEST	BROM-SULF-ALEIN RETENTION, %, 30 MIN.	ALB./GLOB., GM. PER 100 ML. BLOOD	CEPHALIN FLOCCULATION	CHOLESTEROL/ESTERS, MG. PER 100 ML. BLOOD
					Resting Before Test	Maximum During Test	Resting After Test					
115	51	Laennec's cirrhosis, anasarea	+	+	61.8	75.4	71.4	Pos.	40	3.40/4.58	4+	197/112
116	60	Laennec's cirrhosis, anasarea		+	82.6	78.8	65.2	Pos.	56	2.2/3.0	3+	114/56
117	60	Laennec's cirrhosis, ascites ^o	+	+	55.6	79.8	59.2	Pos.		1.9/2.2	2+	120/51
118	75	Subacute yellow atrophy ^a	+	+	65.5	83.0	81.6	Pos.		2.2/4.1	3+	156/88
119	31	Toxic cirrhosis, ascites ^b		+	111.8	91.0	84.0	Pos.	25	2.05/2.87	4+	271/120
120	18	Heart failure	+	+	86.9	92.5		Pos.	32	5.6/1.9		165/106
121	61	Laennec's cirrhosis ^o	+	+	89.9	98.6	81.4	Pos.	49	1.6/3.7	3+	160/119
122	78	Arteriosclerosis, ieterus		+a	98.6	111.0	85.8	Pos.	30	2.7/2.3	3+	111/77
123	53	Laennec's cirrhosis, ieterus	+		89.0	155.0	152.0	Pos.		2.8/3.4	4+	90/75

^a Finding at autopsy.^b Finding on biopsy.^o Finding at operation.

? Doubtful.

+ Clinical evaluation of impaired liver function.

- Clinical evaluation of no impaired liver function.

+a Evidence of impaired liver function associated with advanced age or severe anemia.

TABLE 2

SUMMARY OF FINDINGS IN TABLE 1 WITH REFERENCE TO VITAMIN K TOLERANCE TEST, BROMSULFALEIN AND CEPHALIN FLOCCULATION TESTS AND EVIDENCE OF CLINICAL HEPATIC DISEASE

TESTS AND REACTIONS	CLINICAL LIVER DISEASE		SEVERE ANEMIA OR AGE OVER 75
	Present (+)	Absent (-)	
Vitamin K Tolerance			
Negative.....	2	46	1
Doubtful.....	6	2	2
Positive.....	48	3	13
Bromsulfalein Retention			
Negative.....	18	37	6
Positive.....	24	4	7
Cephalin Flocculation			
Negative.....	21	25	6
Positive.....	28	16	5

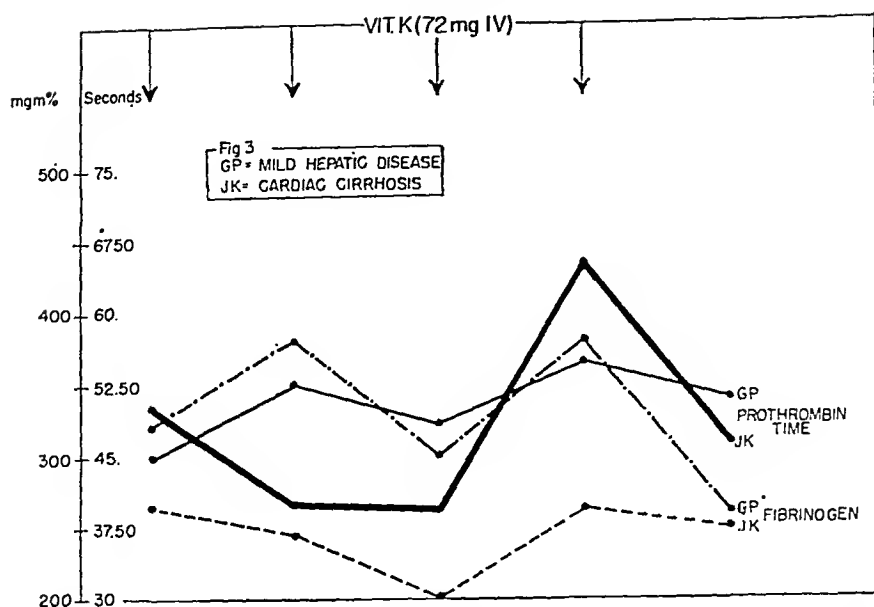


FIG. 3. Serial estimations of prothrombin time of diluted (12.5 per cent) plasma (solid line) and fibrinogen concentration (broken line). Each arrow represents an intravenous injection of 76 mg. of Synkayvite following withdrawal of the blood sample. Patient GP (age 69, Table 3). The prothrombin time shows changes paralleling the fibrinogen concentration. This is the reverse of what should occur if alteration in diluted (12.5 per cent) plasma prothrombin time were due to changes in fibrinogen concentration. Patient JK (age 73, Table 3). The curve shows an initial fall in prothrombin time followed by an increase. The fibrinogen concentration fluctuates independently.

TABLE 3

COMPARISON OF VITAMIN K TOLERANCE TEST WITH OTHER FUNCTIONAL TESTS
AND WITH CHANGES IN FIBRINOGEN CONTENT OF BLOOD

AGE	DIAGNOSIS	PROTHROMBIN TIME (SEC.) 12.5 PER CENT		FIBRINOGEN (MG. PER 100 ML.)			BSP, ^a RETEN- TION 30 MIN.	CEPH. FLOC. ^c	ALB./GLB. ^e	CHOL./ EST. ^e
		Rest- ing	Maxi- mum	Be- fore test	During test	After test				
88	Heart disease	42.6	31.8	410	400	450	10	0	3.40/1.99	250/181
37	Alcoholism, fatty liver	40.8	46.2	311	246	609		0	3.87/2.20	278/216
74	Heart disease	35.4	48.0	210	240 to 370	410	3			
65	Portal cirrhosis	53.6	52.0	500	400 to 510	440	10-20	2+	3.54/3.90	210/128
69	Adenocarcinoma	45.4	54.2	320	300 to 380	240	5		3.73/1.91	165/124
51	Luetic heart disease, malaria*	56.0	56.2	270	310	400	0	4+	3.95/2.10	185/123
58	Portal cirrhosis	49.2	62.6		220 to 300	300	10	2+	4.22/2.29	179/40
73	Cardiac cirrhosis	49.0	64.8	260	205 to 250	220	25	3+	4.24/2.59	130/78
45	Portal cirrhosis	58.2	70.0	360	200 to 300	490	55	2+	4.79/2.54	192/145

^a BSP indicates per cent of bromsulfalein retention at end of 30 minutes.

^c Ceph. Floc. indicates reading of cephalin flocculation test at 48 hours.

^e Alb./Glb. indicates albumin and globulin in Gm. per 100 ml. blood.

^e Chol./Est. indicates cholesterol and cholesterol esters in mg. per 100 ml. blood.

* All patients except this one had enlargement of the liver.

TABLE 4

COMPARISON OF RESULTS OF VITAMIN K TOLERANCE TEST AND OTHER LIVER FUNCTION TESTS WITH FINDINGS IN BIOPSY OF LIVER, AT OPERATION OR AT AUTOPSY

CASE NO.	NAME	AGE	CLINICAL DIAGNOSIS	PATHOLOGIC FINDINGS IN LIVER	VIT. K TOL. TEST	BSP. % RET. 1/2 HOUR	ALB./GLB.	CEPH. FLOC.	CHOL./EST.
5	HD	50	Hypertension ^a	Congestion	Neg.	2		0	
36	MH	55	Polyserositis (?), nephrotic syndrome (?) ^b	No changes	Neg.	0	3.2/2.3	2+	500/431
26	SL	54	Rheumatic heart disease, chronic failure, cardiac cirrhosis ^a	Chronic passive congestion	Neg.	0	4.9/2.4	0	258/141
2	JV	56	Carcinoma tail of pancreas ^a	Few small metastatic nodules	Neg.	0	3.9/2.8	2+	320/134
38	GG	49	Rheumatoid arthritis, amyloidosis ^b	Moderate amyloid infiltration	Neg.	1	1.9/2.9	0	358/170
21	FT	25	Simmond's disease, chronic glomerulonephritis ^a	Liver 760 Gm., mild cirrhosis	Neg.	2	2.1/5.0	2+	161/83
53	JH	37	Alcoholism, fatty liver ^b	Fatty infiltration, and cirrhosis	Dbt.		3.9/2.2	0	278/215
51	AG	78	Chronic cholecystitis, recurrent cholangitis ^{b, c}	Periportal cirrhosis, fibrous, with pericholangitis	Dbt.	7	3.9/2.5	0	178/102
61	RM	68	Rheumatic heart disease, chronic failure, Paget's disease ^a	Cardiac cirrhosis	Pos.	40	2.8/2.0	2+	227/125
58	HG	64	Acute hepatitis, neurosyphilis	Cirrhosis, biliary obstruction	Dbt.		3.1/3.0	4+	182/62
60	DT	49	Cirrhosis	Early cirrhosis, fatty infiltration	Pos.	20	4.75/3.24	0	143/76
90	IB	38	Lacnec's cirrhosis ^b	Portal cirrhosis	Pos.		3.8/2.2	1+	190/163
73	FA	60	Hypertension, chronic failure, cardiac cirrhosis ^a	Chronic passive congestion	Pos.	20	2.9/2.9	3+	198/74

^a Finding at autopsy.

^b Finding on biopsy.

^c Finding at operation.

TABLE 4—Concluded

CASE NO.	NAME	AGE	CLINICAL DIAGNOSIS	PATHOLOGIC FINDINGS IN LIVER	VIT. K TOL. TEST	BSP. % RET. 1 HOUR	ALB./GLB.	CEPH. FLOC.	CHOL./EST.
71	BH	60	Carcinomatosis of liver ^a	Laennec's cirrhosis, extensive malignant hepatoma	Pos.	32	3.0/2.0		167/84
78	GB	68	Recurrent cholangitis, liver abscess ^a	Multiple abscesses	Pos.		2.6/2.6	4+	190/114
77	OJ	58	Portal cirrhosis	Portal cirrhosis	Pos.	45	3.34/2.84	4+	193/119
87	AS	75	Coarctation of aorta ^a	Advanced autolysis	Pos.	32	3.4/3.2	2+	102/51
91	TC	52	Laennec's cirrhosis ^b	Portal cirrhosis	Pos.	2	2.2/2.7	0	208/98
95	PR	76	Subacute hepatitis, recurrent cholangitis ^b	Pericholangitis	Pos.	15	3.0/3.0	3+	200/111
107	ET	71	Banti's syndrome, possible schistosomiasis of liver ^b	None	Pos.	24	4.0/2.7	3+	121/83
108	AE	64	Felty's syndrome ^a	Toxic hepatitis, fatty infiltration, cholelithiasis	Pos.	0	1.9/2.6	2+	118/58
112	JR	61	Laennec's cirrhosis ^b	Portal cirrhosis, fatty metamorphosis	Pos.	1	1.9/3.3	0	114/57
116	BR	60	Laennec's cirrhosis, ^a alcoholism	Portal cirrhosis	Pos.	56	2.2/3.0	3+	114/56
117	JP	60	Laennec's cirrhosis, portal vein thrombosis ^c	Cirrhosis, portal vein thrombosis	Pos.		1.9/2.2	2+	120/51
118	BF	75	Subacute liver atrophy ^a	Subacute yellow atrophy	Pos.		2.2/4.1	3+	156/88
121	TP	61	Alcoholic cirrhosis	Atrophy, fibrosis, nodularity	Pos.	49	1.6/3.7	3+	160/119
123	GT	53	Acute hepatitis, cirrhosis ^a	Portal cirrhosis, acute hepatitis, hepatoma	Pos.		2.8/3.4	4+	90/75
See Table 3	GP	69	Chronic pulmonary disease	Periportal changes, adenocarcinoma	Pos.	5	3.73/1.91		165/124
See Table 3	KF	45	Portal cirrhosis, pulmonary disease	Enlargement	Pos.	55	4.79/2.54	2+	192/145
119	MB	31	Cirrhosis, ascites	Toxic cirrhosis	Pos.	25	2.05/2.87	4+	271/120

in fibrinogen concentration as depicted here, do not affect the results of the prothrombin time estimations by the technic employed in this study.

The data recorded in Table 3 for this group of patients, again demonstrates the greater sensitivity of the vitamin K tolerance test in detecting impaired liver function when compared to both the bromsulfalein retention and the cephalin flocculation tests.

ANALYSIS OF CASES WITH HISTOLOGIC STUDY

The results of the vitamin K tolerance tests were correlated with other liver function tests and liver biopsies in 30 persons. Table 4 gives the data in adequate detail to permit individual analysis of each patient. In 24 of the 30 biopsies there was histologic evidence of liver disease. Of these 24 patients, 2 yielded negative tests. One of these patients had moderate cardiac cirrhosis, and the other had moderate amyloid infiltration of the liver. In 20 of the 24 who were tested for bromsulfalein retention, the test showed abnormal retention in 12 and negative results in 8. Four persons in whom no histologic evidence of liver disease was found yielded normal findings with both the vitamin K tolerance and the bromsulfalein retention tests. The remaining 2 patients, 1 with cardiac cirrhosis and 1 with amyloidosis also gave negative results with both tests. The cephalin cholesterol flocculation test was performed in 28 of the 30 patients. It is of interest to note that of 9 patients in this group with negative cephalin flocculation tests, 6 proved to have cirrhosis histologically. The results obtained in this group of 30 patients with liver biopsies demonstrated that the vitamin K tolerance test is a reliable indicator of liver function.

DISCUSSION

The vitamin K tolerance test reflects the capacity of the liver to produce prothrombin. When considered along with the results of other functional tests, the procedure aids in the evaluation of the over-all functional state of the liver. Many tests have been devised to estimate a particular function of the liver. The most sensitive can be used as the "scout" test in an investigation of liver function. Bromsulfalein retention has enjoyed great popularity as such a test. The results obtained in the present study indicate that the vitamin K tolerance test is more sensitive than the dye test. Others^{10, 11} have found in infectious hepatitis that bromsulfalein retention and bilirubinuria were usually the first to become abnormal in early clinical pre-icteric hepatitis. The abnormal retention of bromsulfalein has been a valuable aid in the detection of other forms of liver disease also. This retention is believed to be dependent upon the functional state of the polygonal cells of the liver. It is known⁸ that extrahepatic tissues are capable of removing injected bromsulfalein from the blood. Consequently, the removal of bromsulfalein appears not to be a specific function of liver parenchyma. In addition, obstruction within the biliary tract or the presence of circulatory stasis may cause abnormal retention of the dye independently of parenchymal impairment. Also, bromsulfalein is foreign to the metabolism of the

liver. The testing of a normal physiologic function would appear preferable to one involving the use of a foreign substance. All of the factors enumerated in regard to bromsulfalein should be considered in evaluating results obtained with the test.^{10, 11}

Neeffe^{10, 11} has found cephalin flocculation, thymol turbidity and colloidal gold tests to be the most sensitive indicators of persistent hepatic dysfunction in cases of infectious hepatitis. Thymol turbidity and cephalin flocculation tests depend upon the presence of beta and gamma globulins respectively.⁵ Hence, serum protein changes referable to disease other than hepatic cellular disturbances, may give rise to positive tests by these methods.

A liver function test which meets the following requirements is most desirable: (1) high degree of sensitivity and reliability and (2) reflection of normal physiologic behavior of the liver parenchyma. These requirements are met by the vitamin K tolerance test as herein described. Its greater reliability and sensitivity as compared to the commonly used procedures have been demonstrated above. Unlike the bromsulfalein retention and the cephalin flocculation tests, the vitamin K tolerance test imposes a "load" upon the prothrombin-producing mechanism, a function that is confined exclusively to the liver parenchyma. The advantage of the procedure is that alterations reflect changes attributable only to disturbed liver function.

It should be borne in mind that prothrombin production can be disturbed in persons having a vitamin K nutritional deficiency such as may result from inadequacy of the vitamin K precursor in the diet or from lack of absorption of the vitamin, as in obstruction to the flow of bile and in diarrheal states. The parenteral administration of vitamin K characteristically is followed within about twenty-four hours by restoration of the prolonged prothrombin time to normal. If there is associated hepatic disease, the hypoprothrombinemia is not corrected.

Fluctuation in fibrinogen level does not influence the prothrombin time as determined by the method employed in this study. The changes in prothrombin time occurring in the vitamin K tolerance test are independent of concurrent serial variations in fibrinogen concentration. This corroborates the clinical impressions previously stated by others.^{2, 7, 13, 15}

Of extreme interest is the further depression in the prothrombin level or activity, induced by parenteral administration of large doses of vitamin K in some cases of liver disease. This is especially striking in cases in which the resting prothrombin time is normal. The explanation for this phenomenon is not clear, but it is believed that in these cases the liver is working at its maximum capacity for this function under the prevailing disease conditions. The added stimulus of massive doses of vitamin K serves only to impair further the prothrombin-producing mechanism. The marked sensitivity of this mechanism suggests that the hepatic reserve for this function is less than that of other known and measurable liver functions. It appears likely that in liver disease, there is a deficient production of the prothrombin substrate. It is also possible that vitamin K combines with this substrate in an abnormal fashion in patients with liver

disease. The removal of substrate in this manner could further reduce the production of prothrombin. It was noted in all cases in which the prothrombin time became prolonged after large doses of vitamin K that the phenomenon was transitory, lasting only twenty-four to forty-eight hours and disappearing with the withdrawal of large doses of vitamin K. The results suggest that in patients with hepatic disease with prolonged resting prothrombin times, vitamin K be given in small doses and repeated only if the prothrombin time does not show further increase. Patients with parenchymatous liver disease without disturbance in absorption of vitamin K can secure augmented prothrombin activity by transfusion of whole, preferably fresh, blood. Where the latter is not available, the administration of frozen or lyophilized plasma may be of aid.

There is no desire to convey the impression that the vitamin K tolerance test, despite its high degree of sensitivity and specificity, can be used by itself to establish the presence or absence of liver disease. For the present, it may be employed as the "scout" test, but it should be correlated with all other means of study and other appropriate functional tests to estimate the functional state of the liver. Biopsy may be of value in doubtful cases.

The vitamin K tolerance test has not been used long enough to conclude whether a particular type of response is of prognostic value. This evaluation can be made only by repeated tests in a large series of cases over an extended period of time. The existence of apparatus to measure prothrombin activity in nearly all clinical laboratories permits the application of the vitamin K tolerance test on a large scale for the estimation of hepatic function.

SUMMARY

1. A standardized vitamin K tolerance test has been used for estimating hepatic function.
2. Excellent correlation has been obtained in results of this test with clinical and histologic findings.
3. The vitamin K tolerance test has been demonstrated to be a sensitive indicator of the functional state of the liver.
4. It is proposed that this procedure be used as a "scout" test for the detection of liver impairment.

Acknowledgments. This work was aided by grants from the Blood Transfusion Association of Greater New York and Hoffman-La Roche, Inc., Nutley, New Jersey. The Thromboplastin used in this study was supplied by the Maltine Company, Morris Plains, New Jersey. The technical assistance of Miss Shirley Schwalb and Miss Yetta Porisowska is gratefully acknowledged.

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ELECTRON MICROSCOPIC STUDIES OF GLOBULAR PROTEINS IN CEREBROSPINAL FLUID*

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The determination of the total protein content of cerebrospinal fluid is one of the most useful diagnostic laboratory procedures in diseases of the nervous system. The increase in total protein of the spinal fluid in meningitis is believed to be the result of transudation of serum through the walls of inflamed blood vessels while in other conditions, such as brain tumor or polyneuritis, the reason for the elevated protein content is not clear.

There are few data available regarding the composition of the cerebrospinal fluid proteins, chiefly because the small amounts of protein present make chemical studies difficult. By the use of the electrophoretic method, Kabat, Moore and Landow¹ have been able to demonstrate variations in the percentage of albumin and different globulin fractions in the cerebrospinal fluid. Since large amounts of fluid, from 70 to 80 ml., are required for this method, its usefulness for clinical purposes is limited.

No reports have been found concerning studies of cerebrospinal fluid proteins with the electron microscope. Since the electron microscope permits observation and photographing of protein particles and direct determination of their size and shape, even with the smallest samples of fluid, we applied this method to a number of normal and abnormal spinal fluids.

MATERIAL AND METHOD

Fresh cerebrospinal fluids from 50 patients were studied in our laboratory and the electron microscopic observations were correlated with the clinical data and the routine laboratory findings (Table 1). The preparation of the specimens for examination with the electron microscope was extremely simple. A drop of undiluted cerebrospinal fluid was placed with a fine pipet on a collodium film supported on a 200-mesh wire screen. The sample was allowed to dry in the air and kept in a desiccator over calcium chloride until ready for examination. The electron micrographs which were photographed on Eastman Contrast Lantern Slides were originally magnified 5,000 times. The size and shape of the globules of protein were studied and outlined on paper placed in a Spencer Microfilm Reader which magnifies 15 times. Thus a final magnification of 75,000 was obtained.

RESULTS

In the micrographs of all fluids we noticed globules which were also present after washing the dried sample of spinal fluid with distilled water. They were absent in the filtrate of spinal fluid half-saturated with ammonium sulfate; therefore, these globules were apparently globulins.

* Received for publication, August 10, 1948.

TABLE 1

CASE	AGE	SEX	CLINICAL DIAGNOSIS	CELL COUNT	PRO-TEIN	WASSER-MANN TEST	COLLOIDAL GOLD TEST	AVERAGE DIAMETER OF GLOBULIN
A. CEREBROSPINAL FLUIDS WITH GLOBULIN PARTICLES OF UNIFORM SIZE								
					mg. per 100 ml.			milli-microns
1	45	F	Psychoneurosis	2	15	neg.	neg.	39
2	2 mo.	M	Hydrocephalus	7	16	neg.		40
3	41	F	Manic psychosis	4	41			40
4	12	M	Myositis	0	15	neg.		40
5		M	Character dissociation					47
6	16	F	Idiopathic epilepsy	5	25	neg.	neg.	45
7	48	F	Climacteric depression	1	36	neg.		53
8	57	M	Idiopathic epilepsy	3	39	neg.	neg.	50
9	5 mo.	F	Respiratory infection	12	30	neg.		53
10	2 wk.	M	Pyloric stenosis	13		neg.		66
11		F	Manic depression					67
12	38	M	Melancholia	3	38	neg.	neg.	68
13		M	Chronic alcoholism	3	63	neg.	1112222100	70
14	65	M	Psychosis	1	35	neg.	neg.	70
15	67	M	Prostatic hypertrophy			1 plus	neg.	67
16	31	F	Treated tertiary syphilis			neg.	neg.	80
17	30	F	Anxiety neurosis	2	39	neg.	neg.	100
18	10	M	Poliomyelitis	72	102	neg.	neg.	110
19		M	Peripheral neuritis	1	38	neg.	1111000000	106
20	75	M	Posttraumatic headache	1	53	neg.	neg.	190
21	20	F	Metastatic chorioepithelioma	3	19	neg.	neg.	100
22	7	M	Posttraumatic headache	2	27	neg.	neg.	130
23	14	F	Convulsions	5	37	neg.	neg.	120
24	55	F	Gumma	3	25	neg.		240
25	55	F	Barbiturate poisoning	2	38	neg.	neg.	240
B. CEREBROSPINAL FLUIDS WITH MARKED VARIATION IN SIZE OF GLOBULIN PARTICLES								
26		M	Chronic alcoholism	3	38	neg.	neg.	50
27	29	F	Encephalitis		38	neg.	neg.	100
28	21	M	Psychopathia	1	31	neg.	neg.	100
29	61	M	Cerebral accident	3	50			150
30	15 mo.	F	Posttraumatic convulsions	0	16	neg.	neg.	175
C. CEREBROSPINAL FLUIDS WITH CLUSTERS OF GLOBULIN PARTICLES								
31	17	M	Encephalitis	160	52	neg.	neg.	53
32	67	F	Psychosis	2	38	neg.	neg.	55
33	34	F	Multiple sclerosis	5	26	neg.	4433320000	55
34		M	Multiple sclerosis					56
35		F	Multiple sclerosis	3	20	neg.	0001110000	50
36	41	F	Encephalitis	56	131	neg.	2233322000	55
37	64	M	Hypertension	1	32	1 plus		67
38		M	Encephalitis	14				93
39		M	Meningovascular syphilis	28	100	4 plus	0012333211	136
40	61	F	Neurosyphilis	1	53	4 plus	2222211000	138

TABLE 1—*Concluded*

CASE	AGE	SEX	CLINICAL DIAGNOSIS	CELL COUNT	PRO-TEIN	WASSER-MANN TEST	COLLOIDAL GOLD TEST	AVERAGE DIAMETER OF GLOBULIN
D. CEREBROSPINAL FLUIDS WITH LARGE PROTEIN AGGREGATES								
41		M	Anxiety neurosis	1	mg. per 100 ml.	neg.	2333432100	80
42	32	F	Acute multiple sclerosis	2	25	neg.	0001110000	107
43		M	Hysteria	0	26	neg.	1344442100	110
44	21	F	Guillain-Barré syndrome	2	150	neg.	0111100000	106
45	35	M	Epidural abscess	710	65	neg.		150
46		M	Hysteria	1	63	neg.	1233332100	160
47	64	F	Subdural hematoma	3	18	neg.	neg.	187
48	39	M	Dementia paralytica	34	75	4 plus	5555542000	215
49	30	F	Latent neurosyphilis	33	156	4 plus	4555420000	240
50	15 mo.	F	Influenza bacillus meningitis	150	206	neg.		175

In normal spinal fluids, the diameter of the protein particles varied from 40 to 60 millimicrons. The particles were spherical and were well separated one from the other. In spinal fluids from nervous diseases, several alterations could be noted. In many cases, the size of the protein particles was larger, reaching a diameter of 240 millimicrons in some, in other cases, in spite of the small size of the individual particles, clusters or larger aggregates were present. There was no definite correlation found between the size of the protein globules and the total protein content of spinal fluid. Diameters as high as 220 millimicrons were seen in fluids in spite of a normal total protein content. While we believe that the colloid particles of various sizes which we saw in the electron microscope represent vital conditions in contradistinction to the general belief that in body fluids the proteins are dispersed in molecular solution, we are less sure about the clusters and larger aggregates seen in some of our cases.

During the drying of the drop of spinal fluid on the collodium membrane, the fluid becomes saturated with sodium chloride and precipitation of protein with formation of aggregates may occur at this time. However, even if clusters and aggregates are artefacts due to our method of preparation, their presence may still be of pathognomonic significance, because normal fluids and many abnormal fluids did not present these aggregates. Formation of clusters, as seen in some of our samples, has been studied in the creaming process of rubber latex (Van

FIG. 1. Cerebrospinal fluid from a 16 year old girl with idiopathic epilepsy. Globulin particles have an average diameter of 45 millimicrons. $\times 20,000$.

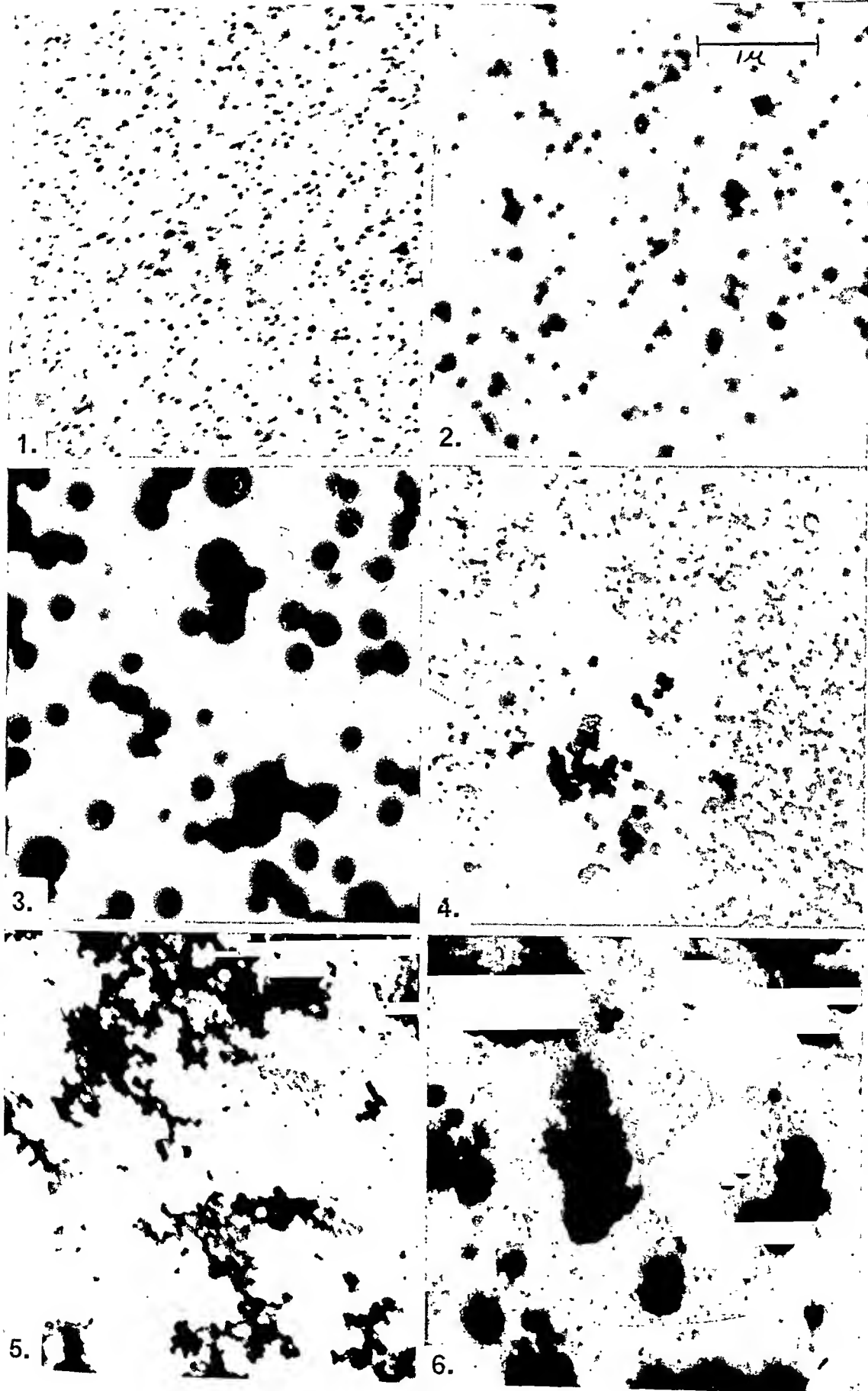
FIG. 2. Cerebrospinal fluid from a 67 year old man in uremia. The size of the protein globules varies between 60 and 100 millimicrons.

FIG. 3. Large globules of protein in fluid from a 17 year old girl with cephalgia. The average diameter is 220 millimicrons.

FIG. 4. Cluster formation of small sized protein particles from a patient with encephalitis.

FIG. 5. Cluster formation of small protein globules from a patient with long-standing multiple sclerosis.

FIG. 6. Large aggregates of protein particles in the cerebrospinal fluid from a 39 year old man with general paresis.



FIGURES 1-6.

Gils and Kraay²). By lowering their stability and adding a creaming agent, the protein particles of rubber latex are made to cohere in clusters which is a reversible process. Coalescence of rubber particles to large irreversible aggregates is observed if the creaming agent is omitted. Of the 13 fluids in which we observed formation of clusters, 12 were definitely pathologic, three of the fluids in this group coming from patients with multiple sclerosis and three from patients with encephalitis. It is of interest that in these fluids, the size of the globulin particles was within normal range. The larger aggregates were observed almost entirely in fluids with increased total protein.

Several patients who had a clinical diagnosis of a functional nervous disorder had cerebrospinal fluid which showed an unusual size or arrangement of protein globules. For instance, the protein globules in the fluid from a patient with cephalgia had a diameter of 220 millimicrons, while a patient with hysteria and a total protein of 63 mg. per 100 ml. of fluid, had protein particles measuring 160 millimicrons. The positive colloidal gold test apparently does not depend on the size of the protein particles, since in two patients with general paresis and multiple sclerosis the gold test was strongly positive in spite of the great difference in the size of the protein globules (Figs. 5 and 6).

Our series of cerebrospinal fluids studied with the electron microscope is much too small to permit definite conclusions as to whether this instrument will be of clinical value in the diagnosis of nervous diseases. We feel, however, that the great variety of patterns which we observed in our material cannot be without biologic significance. It may well be that the examination of the colloidal properties of the spinal fluid proteins will become as useful a test as the determination of sedimentation rate of erythrocytes in blood.

CONCLUSIONS

Fifty cerebrospinal fluids were studied with the electron microscope.

In all fluids, globulin particles of spherical form and measuring from 40 to 240 millimicrons in diameter could be seen. This would indicate that the globular proteins in cerebrospinal fluid are not dispersed in molecular solution, as generally believed, but exist as molecular aggregates.

Fluids from patients with nervous diseases often showed increase in the size of the individual protein particles or formation of clusters and aggregates.

The study of the size and arrangement of protein particles in cerebrospinal fluid with the electron microscope promises to be of value, not only from the clinical standpoint, but also in fundamental research of protein chemistry.

Acknowledgment. These studies were made in the Electron Microscope Laboratory of St. Francis Hospital which was installed by the Kansas Division of the American Cancer Society.

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THE EFFICIENCY OF BLOOD SUBSTITUTION*

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The pathologic basis for blood substitution and the indications for its application in the treatment of erythroblastosis fetalis have been previously described.⁸⁻¹⁰ Since the initial report of a group of cases successfully treated by this method,⁸ widespread adoption of the principle has led to various modifications in technic. Three different routes, namely, the sagittal sinus, the radial artery and the umbilical vein, have proved most useful. It is the experience of one of us (W) that the performance of all three techniques are of equal facility and with proper care are equally free from complication. It is recommended that physicians, who may find it necessary to perform blood substitution, particularly in the newborn, familiarize themselves with the three methods, since individual variations might occasionally make one or two of these sites unavailable.

A continuation of the study of blood substitution suggested that additional pertinent information was required in order to obtain the greatest mathematical efficiency in the smallest period of time. If it were possible to exsanguinate a patient completely in a single procedure and then infuse an equal amount of new blood, a 100 per cent substitution would result. The impracticability of such an attempt is apparent. On the other hand, if it were possible to continue a simultaneous or alternating withdrawal and replacement for a long enough period of time, while maintaining a constant volume, an almost complete substitution could be effected. Experience has shown that in a six pound baby, a two volume substitution can be performed in approximately one hour. A condition almost equivalent to complete substitution by simultaneous infusion and withdrawal will occur mathematically after five blood volumes have been exchanged. If no unusual technical difficulties, such as clotting or reactions to citrate or hypocalcemia, occur as a result of an attempted exchange with five volumes of blood, the time required would be approximately two and one-half hours. Such a prolonged procedure should be avoided if the desired results can be obtained otherwise.

In order to determine the most efficient procedure commensurate with therapeutic requirements, the following questions must be answered:

A. What is the minimum percentage of substitution that will protect the infant?

B. What modification of the substitution technique will provide this minimum amount of blood most rapidly and with an adequate margin of safety?

A. THE MINIMUM PERCENTAGE OF SUBSTITUTION WHICH WILL PROTECT THE INFANT

Complete substitution cannot be obtained by any of the means now in use without subjecting the patient to shock or other untoward reaction. What,

* Received for publication, March 30, 1948.

then, may be considered an adequate substitution beyond which it is not necessary to continue the procedure? It is known that a certain amount of hemolysis occurs in all newborn infants.¹² This is probably the result of the change-over from the relatively low oxygen tension in intra-uterine life to the relatively higher oxygen tension in postnatal existence. Attempts to relate postnatal blood destruction within physiologic limits to the fragility of the fetal erythrocytes have not been entirely successful. Studies of fragility of erythrocytes in newborn infants have resulted in conflicting reports, indicating increased resistance,⁵ apparently normal resistance⁷ and increased fragility.³ Perhaps the findings of Waugh and associates¹¹ that there is a wide spread (aniso-hemolysis) with numerous cells having increased fragility as well as many cells having increased resistance, may explain why from 15 to 25 per cent of the normal infant's erythrocytes are destroyed in the first few days of life. Whatever the reason, it is known that there is an elevated icterus index in all neonatal infants, with or without visible jaundice, and the high erythrocyte count of intra-uterine existence falls rapidly after birth. The fact that so-called physiologic jaundice is more common in premature or immature infants is an indication that visible icterus occurs only if the excretion of bilirubin is slowed in a poorly functioning liver. It has been shown⁶ that experimental hemolysis in dogs would not produce visible icterus in the presence of competent liver function. Dameshek and co-workers¹ have also shown that there is an increased hemolytic index in newborn normal infants. Apparently, the destruction of up to 25 per cent of the initial erythrocyte volume will not in itself embarrass normal liver function and in a premature infant will cause only mild visible jaundice. In erythroblastosis fetalis where, because of the inherent disturbance, the functions of the liver may be considerably less efficient,^{2, 10} it is believed that protection of the liver will be achieved by reducing possible blood destruction to below 25 per cent, with an added margin of safety if the hemolyzable residue is reduced to about 10 per cent. This also provides an excess of viable red cells to cover the destruction of the remaining 10 per cent. In actual experience, this low figure is not always required. The infants treated early in this investigation recovered following substitutions which left a residue of from 25 to 30 per cent Rh-positive blood. However, it would seem sounder practice to continue transfusion until a 90 per cent substitution has been accomplished, since it is not always possible to predict the rapidity of hemolysis or the efficiency of liver function. This is especially true if such a 90 per cent result can be obtained with relative ease.

B. MODIFICATIONS OF SUBSTITUTION METHODS TO INTRODUCE 90 PER CENT NEW BLOOD

Alteration of the efficiency of substitution can be achieved irrespective of the technic or route used for the procedure. As previously stated, the removal and subsequent infusion of a total volume would represent a 100 per cent substitution. Thus, by altering the amounts or the speed of each step in the method it is possible to affect the percentage of original blood remaining. In this manner substitution technics can be modified in the following ways:

1. Continuous substitution transfusion with equal volume replacements
2. Intermittent, or discontinuous, substitution transfusion with equal volume replacements
3. Partial initial exsanguination, followed by
 - a. Continuous substitution transfusion, or by
 - b. Intermittent substitution transfusion
4. Partial initial increment of new blood, followed by
 - a. Continuous substitution transfusion, or by
 - b. Intermittent substitution transfusion
5. Continuous substitution transfusion with unequal volume replacements
6. Intermittent substitution transfusion with unequal volume replacements.

1. Continuous Substitution Transfusions with Equal Volume Replacements

If v_1 cc. of Rh-negative blood is added per unit time to v_0 cc. of Rh-positive blood, and if mixing is assumed to be instantaneous, and if v_1 cc. of the mixture is removed per unit time, the resulting concentration of Rh-positive blood at any instant is given by the differential equation:

$$(1a) \quad \frac{d(C+)}{dt} = -\frac{v_1(C+)}{v_0}$$

(Where $C+$ stands for concentration of Rh-positive blood). Integrating and using the limits, where $t = 0$, $C+ = 1$ or 100 per cent, we get

$$(1b) \quad C+ = e^{-(v_1 t / v_0)}$$

where e is the Naperian base and $v_1 t$ is the volume of Rh-negative blood used. According to this formula, and as is shown in Figure 1, a semi-logarithmic plot of the formulas as a straight line A, a total volume replacement leaves a remainder of 37 per cent of the original blood, a two volume substitution 13.5 per cent, a three volume substitution 5 per cent, and a four volume substitution 1.8 per cent.

The technic of such a substitution involves the control of bleeding and replacement to the extent that blood enters through one channel at the same rate and in equal-sized drops as that removed from another vessel. In practice this is difficult to maintain for the extent of a complete volume substitution or more than one volume substitution. Even if slight variations occur during the procedure, there is only slight influence on the efficiency of the percentage change. The continuous, drop by drop substitution is represented by the technic of permitting blood to drip out of the cut or cannulated radial artery at a rate equal to the introduction into a cannulated superficial vein.

2. Intermittent Substitution Transfusion with Equal Volume Replacements

Let the initial concentration of Rh-positive blood equal $C+$ or 100 per cent. If v_0 is the original volume and v_1 cc. is removed and replaced by v_1 cc. of Rh-negative blood, then the new concentration $C_1+ = 1 - \frac{v_1}{v_0}$. If the process is repeated and v_1 cc. of the mixture is removed and replaced by v_1 cc. of Rh-negative blood, we get

$$(2a) \quad C_2+ = \left(1 - \frac{v_1}{v_0}\right)^2$$

If n steps are taken, we get:

$$(2b) \quad C_n + = \left(1 - \frac{v_1}{v_0}\right)^n,$$

where $\frac{v_1}{v_0}$ represents the fraction of blood volume substituted per step.

With this technic, a measured volume of blood is removed by syringe and an equal volume of Rh-negative blood is then given either by syringe or by gravity

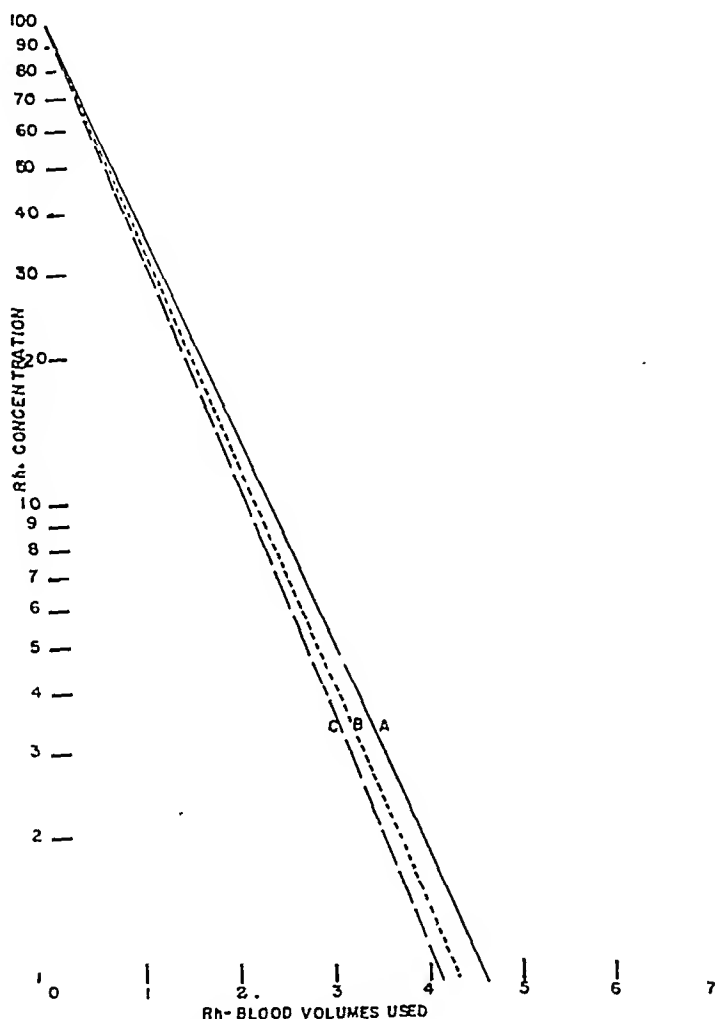


FIG. 1. A represents equation (1b) of text, continuous substitution transfusion; B represents equation (2b) of text, intermittent substitution transfusion, in 0.1 volume steps; C represents equation (2b) of text, intermittent substitution transfusion, in 0.2 volume steps.

drip, and the procedure is repeated until the desired substitution is obtained. By this method the alternate withdrawals and replacements may be performed through a single vessel. The only vessel we have found adaptable to this use is the umbilical vein. With slight variation, however, the same method may be applied through two vessels, if controlled bleeding by syringe can be maintained.

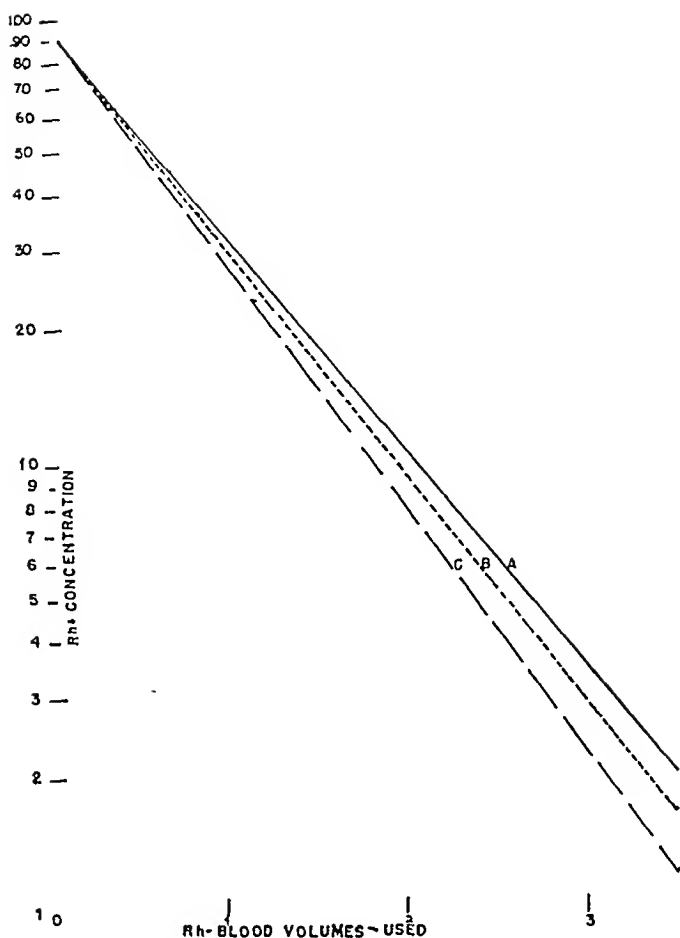


FIG. 2. A represents equation (3b) of text, 0.1 volume removed first, then followed by continuous substitution transfusion, with ultimate restoration to original volume by the addition of 0.1 volume of Rh-negative blood. Value $\frac{v_1}{v_0}$ equals 0.1 in graph. B represents equation (4a) of text, 0.2 volume removed first, and 0.1 of remaining volume added as Rh-negative blood, and the same volume added as is subtracted from the mixture. After the last extraction, the blood volume is restored by the addition of Rh-negative blood. In the graph, the value $\frac{v_1}{v_0} = \frac{v_2}{v_r} = 0.1$, and the value $\frac{v_r}{(v_r + v_2)} = \frac{10}{11}$. C represents equation (4b) of text, 0.1 volume removed first, followed by an additional removal of 0.1 of the remaining volume. An equivalent of the latter removal is added as Rh-negative blood, and an identical equivalent of the mixture is removed and replaced by Rh-negative blood; and so on. Ultimately the blood volume is restored by the addition of the Rh-negative blood. In the graph, $\frac{v_1}{v_0} = \frac{v_2}{v_r} = 0.1$.

In our experience, the only vessel permitting such controlled bleeding by syringe is the superior sagittal sinus. The infusion may then be given through any cannulated vein. A slight mathematical aberration is introduced by the neces-

sity of maintaining infusion while blood is withdrawn. For those few seconds the procedure approximates the continuous type of substitution, but this is compensated by the slightly increased rate of removal by syringe.

The efficiency of the intermittent type of substitution is altered by the amount

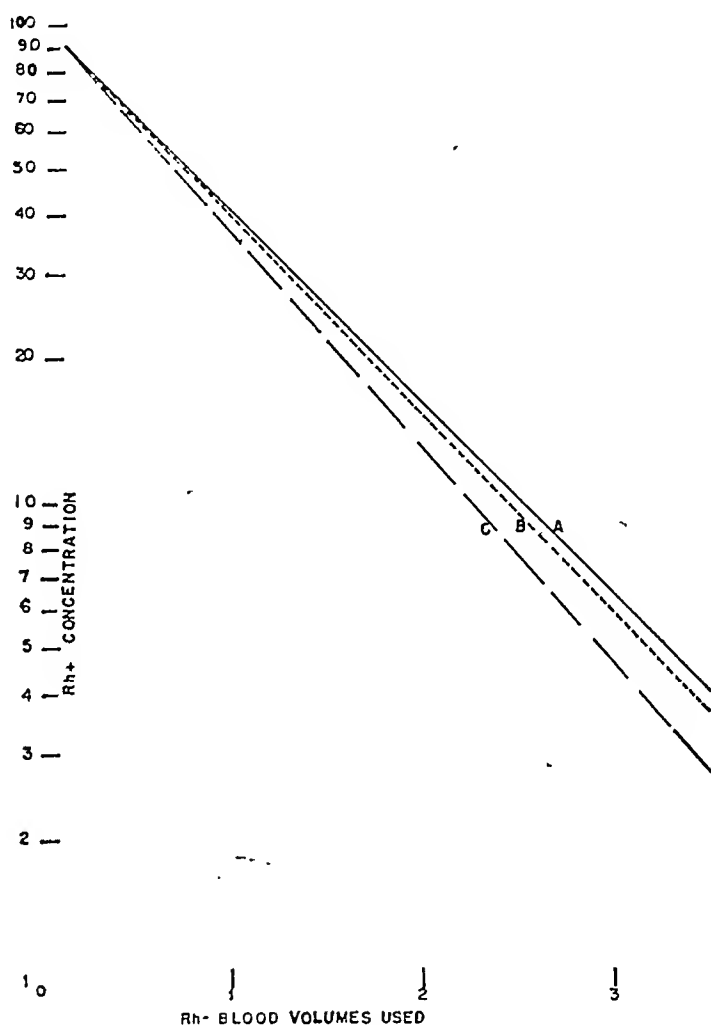


FIG. 3. A represents equation (4c) of text, $\frac{v_0}{(v_0 + v_1)} = \frac{10}{11}$, and $\frac{v_2 t}{v_s}$ represents the additional Rh-negative blood added. B represents equation (4d) of text, $\frac{v_0}{(v_0 + v_1)} = \frac{10}{11}$ and $\frac{v_2}{v_s} = 0.1$. C represents equation (4d) of text, $\frac{v_0}{(v_0 + v_1)} = \frac{10}{11}$ and $\frac{v_2}{v_s} = 0.2$.

of blood alternately removed and replaced. The larger the quantity in each step, the more efficient the substitution, but there are technical limitations which must be considered. If we assume an original blood volume of approximately 250 cc., then by using a 50 cc. syringe, one can perform the substitution in 0.2 volume steps. If 0.1 volume steps are used the procedure is slightly less efficient (Fig. 1, curves B and C). Thus, with 0.2 volume steps, at the end of a 2-volume

substitution there will remain 10.5 per cent of the original blood, while with 0.1 volume steps, 11.5 per cent will remain.

3. *Partial Initial Exsanguination Followed by Continuous or Intermittent Substitution*

If the initial volume is v_0 , and v_1 cc. are *first* removed, leaving the blood volume $v_0 - v_1 = v_r$, and v_2 cc. of Rh-negative blood are added per unit time, and v_2 cc. of the resulting mixture are removed per unit time, we get from equation (1a)

$$\text{Rh-positive } + = e^{-(v_2 t / v_r)}.$$

If v_1 cc. of additional Rh-negative blood is added to restore the initial volume, we get

$$(3a) \quad C+ = \left(1 - \frac{v_1}{v_0}\right) e^{-(v_2 t / v_r)}.$$

In particular, if the value $\frac{v_1}{v_0} = \frac{v_2}{v_r}$, then

$$(3b) \quad C+ = (1 - a)e^{-at}, \quad \text{where } a = \frac{v_1}{v_0}.$$

For the analogous intermittent procedure: If v_1 cc. is *first* withdrawn from the original volume v_0 , leaving $v_r = v_0 - v_1$, and if v_2 cc. of Rh-negative blood is then added and v_2 cc. of the resulting mixture is subtracted, and the procedures repeated until having subtracted the last v_2 cc. of the mixture, v_1 cc. of Rh-negative blood is added to restore the initial volume, we get

$$(4a) \quad C+ = \left(1 - \frac{v_1}{v_0}\right) \left(\frac{v_r}{v_r + v_2}\right)^n.$$

If the procedure is slightly modified in that v_1 cc. is *first* withdrawn and then an *additional* v_2 cc. is subtracted, followed by infusion of v_2 cc. of Rh-negative blood, and the latter steps repeated until ultimate restoration of the original volume is made by the addition of v_1 cc. of Rh-negative blood, we get

$$(4b) \quad C+ = \left(1 - \frac{v_1}{v_0}\right) \left(1 - \frac{v_2}{v_r}\right)^n.$$

In particular, when $\frac{v_1}{v_0} = \frac{v_2}{v_r}$, we get $C+ = \left(1 - \frac{v_1}{v_0}\right)^{n+1}$

An examination of Figure 2 shows that the intermittent procedure is more efficient than the analogous continuous one. As originally reported,⁹ it was the practice to first remove approximately 0.2 of the blood volume and then to continue with the substitution either by continuous or intermittent technic. In effect, this reduces the initial volume, and the subsequent steps remove proportionately larger quantities of the original erythrocytes. By this method a 90 per cent substitution can be accomplished by a 1.8 volume procedure. This represents a saving in time of from fifteen to twenty minutes and is a definite advantage.

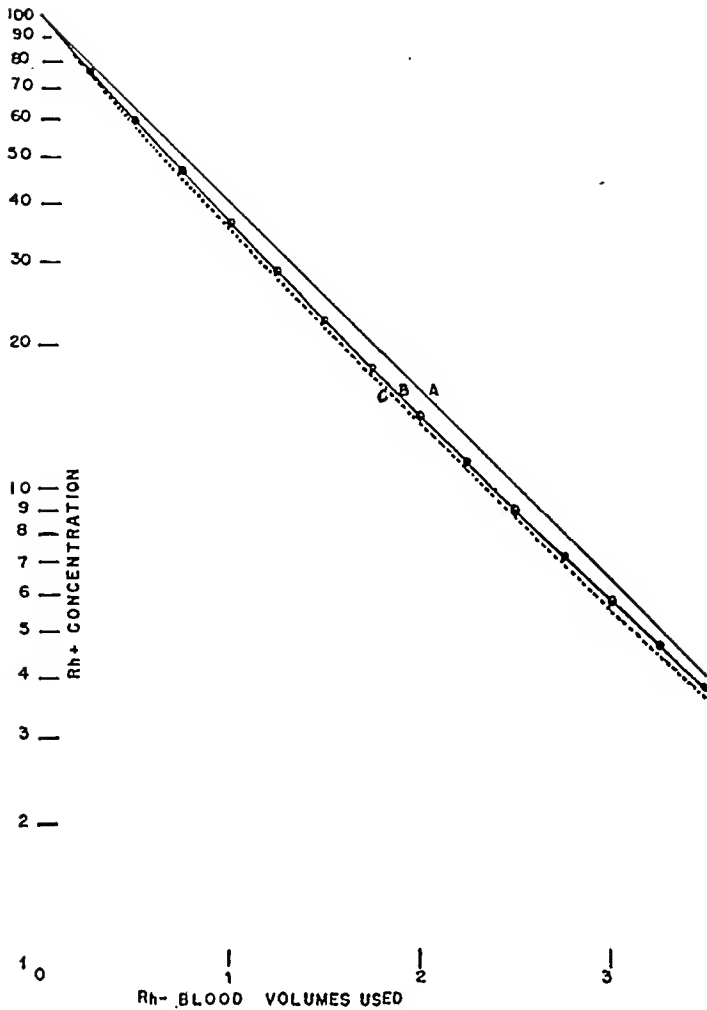


FIG. 4. A represents equation (5b) of text, $\frac{v_o}{v_t} = 1.2$, $v_1 = 3$ blood volumes of Rh-negative blood, and $v_f - v_0 = 1.2 - 1.0 = 0.2$. B represents equation (6) of text, $\frac{v_1}{v_0} = 0.0933$ and $\frac{v_2}{v_0} = 0.1$ and transfusion is accomplished in 30 steps using a total of 3 blood volumes of Rh-negative blood and ending with 1.0 v_0 volumes. C represents equation (6) of text, $\frac{v_1}{v_0} = 0.1$ and $\frac{v_2}{v_0} = 0.12$ and the transfusion is accomplished in 25 steps using a total of 3 blood volumes of Rh-negative blood and ending with 1.2 v_0 volumes.

4. *Partial Increment of Rh-Negative Blood Followed by Continuous or Intermittent Substitution*

If the initial volume is v_0 , and v_1 cc. of Rh-negative blood is *first* added, the new volume is $v_0 + v_1 = v_s$. If v_2 cc. of Rh-negative blood is then added per unit time, and v_2 cc. of the resulting mixture is removed simultaneously per unit time, we get

(4e)
$$C+ = \frac{v_0(e^{-(v_2t/v_s)})}{v_0 + v_1}$$

If, at the end of the procedure, v_1 cc. of the mixture is removed to restore the initial volume, the concentration of Rh-positive blood per cc. does not change, but the total quantity of Rh-positive cells will be lessened.

For the intermittent analogue: If the new volume $v_s = v_0 + v_1$, and if v_2 cc. of the mixture is removed, followed by the addition of v_2 cc. of Rh-negative blood, we get

$$(4d) \quad C+ = \frac{v_0 \left(1 - \frac{v_2}{v_s}\right)^n}{v_0 + v_1}$$

The graphs for equations (3c) and (4d) are given in Figure 3.

Wiener¹³ has altered the procedure by first giving the infant approximately 0.2 volumes of Rh-negative blood and then proceeding with a continuous type of substitution. The disadvantages of this technic are apparent when one realizes that this immediately increases the blood volume and dilutes the amount of original blood subsequently removed. By this technic, in order to reach 90 per cent efficiency, it is necessary to continue the procedure until at least 2.5 volumes have been substituted, thereby prolonging it by about twenty-five minutes. There are, of course, occasional instances where the infant is born with a severe anemia, which would require this technic in order to bolster the circulation. These cases, however, are the exception rather than the rule.

5 and 6. Substitution with Unequal Volume Replacements

If v_1 cc. of Rh-negative blood is added per unit time to v_0 (the initial volume) and v_2 cc. of the mixture is removed per unit time (v_1 does not equal v_2), the concentration of Rh-positive blood remaining after time, t , is given by the formula

$$(5a) \quad \frac{d(C+)}{dt} = - \frac{v_1 C+}{v_0 + (v_1 - v_2)t}$$

Integrating and substituting limits (when $t = 0$, $C+ = 1$) we get

$$(5b) \quad C+ = \left(\frac{v_0}{v_0 + (v_1 - v_2)t} \right)^{(v_1/v_1 - v_2)} = \left(\frac{v_0}{v_f} \right)^{(v_1/v_f - v_0)}$$

where v_i = total number of cc. of Rh-negative blood introduced and v_f = the final volume in cc.

If v_f is less than v_0 , then formula (5a) becomes on restoration to original volume

$$(5c) \quad C+ = \left(\frac{v_f}{v_0} \right) \left(\frac{v_f}{v_0} \right)^{(v_1/v_0 - v_f)}$$

The analogous intermittent case leads to a π function given in the following formula:

$$(6) \quad C+ = \prod_{n=1}^{n=\pi} \left[\frac{v_0 - nv_1 + (n-1)v_2}{v_0 - n(v_1 - v_2)} \right]$$

where the symbol π means that every fraction (after having substituted for n the numbers 1, 2, 3, etc.) is multiplied by every other fraction. If the final volume is less than the original volume, the above formula is multiplied by $\frac{v_f}{v_0}$.

In practice, these last two methods would be most difficult to maintain as a technic. They have been computed as of theoretical interest only. The results are given in Figure 4. Again, it should be pointed out that the intermittent technic yields more efficient results.

Within recent years the principle of blood substitution has been applied most consistently in the therapy of erythroblastosis fetalis. In the past, it has been used in other toxic states, such as burns in small infants, and in adults in severe

toxemias, as uremia, septicemia and gas poisoning. The above data will apply in all cases, and the procedure will be as effective as desired if allowance is made for the original estimated blood volume. In the average adult, a substitution by 0.2 volume steps would require removal and replacement by 1,000 cc. at a time, and it might, therefore, be best to employ 0.1 volume steps. The continuous simultaneous procedure can be performed in adults without regard to the size of the steps, but is correspondingly less efficient. This, however, can be compensated by prolonging the procedure, since this is more feasible in adults than in children or infants.

SUMMARY

1. The efficiency of substitution transfusion is estimated mathematically and the variations in efficiency by different procedures are illustrated.
2. The minimum requirement of substitution in erythroblastosis fetalis plus the required margin of safety is estimated on the basis of the average amount of hemolysis in the normal newborn infant.
3. The optimum percentage of substitution in erythroblastosis fetalis is suggested as being 90 per cent.
4. The efficiency of the substitution as performed in the three commonly used technics is calculated. In order to arrive at 90 per cent efficiency in bleeding from the radial artery, 2.5 volumes of blood must be substituted, and in bleeding from the sagittal sinus or umbilical vein, 1.8 volumes of blood must be substituted.
5. The sagittal sinus or umbilical vein technics are approximately 33 per cent more efficient than the radial artery route.

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ADVANCES IN HISTOPATHOLOGIC TECHNIC*

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Histopathologic technic is a subject over which many have been concerned during the past century and a quarter since iodine was first used as a histochemical reagent in the study of the structure of the starch grain by Caventou in 1826. Carmine was introduced into histology about 1850; and I can recall that it was still used as a single stain for embryos when I was an undergraduate. Fuchsin and hematoxylin appeared in histology in 1863 and 1865, double staining with carmine and picric acid in 1867, and in the same year Perls applied the ferrocyanide reaction for the histochemical demonstration of iron.

Prominent in the advances in histologic technic as applied to pathology were Carl Weigert and Paul Ehrlich in the late 19th century. Weigert was an exponent of the experimental trial and error method, Ehrlich of the theoretical approach. The following story was told to me by one of Weigert's students. The Herr Professor had just demonstrated one of his methods and the students clustered about him after the class. One of them asked, "But, Professor, what is the theory of this method?" Characteristically, Weigert replied, "Ach! die Theorie! You should ask mine nephew, Paul, about that", referring to Ehrlich.

Methods for fibrin, for myelin, for elastic tissue were introduced by Weigert and still survive, as well as a number of formulas, such as his iron hematoxylins, his anilin xylene, his borax ferricyanide, his carbol xylene, his permanganate-oxalic acid bleach, his myelin mordants and his variant of the van Gieson stain, which are still in familiar use.

Ehrlich's greatest contribution was again, characteristically, theoretical. He introduced the still-prevailing concepts of classification of leukocyte granules into oxyphil, neutrophil and basophil, although the triacid and dahlia stains which he devised are memories only, having been supplanted largely by the azure eosin technics of Romanovsky, Nocht, Giemsa, Wright, Leishman and a host of others.

Another great service of Ehrlich's to histopathologic technic was his compilation and editing, with Krause, Mosse, Rosin and Weigert, of the *Encyklopädie der mikroskopischen Technik* to which 60 authors contributed. Among these we remember Benda and Heidenhain for their iron hematoxylins, Blum for his introduction of formaldehyde, Nissl for the tigroid stain, Unna for mast cell and methyl green pyronin, Nocht and Pappenheim for blood stains and Mayer for carmine and hematoxylin formulas.

About this time, 1897, Mallory and Wright brought out the first edition of the

* Presented at the meeting of the North Central Section of the College of American Pathologists in Milwaukee, May 10, 1948. Received for publication, May 21, 1948.

well known *Pathological Technique* which went through 8 editions to 1924 and was followed by Dr. Mallory's last book with the same title in 1938. Schmorl's first edition of his *Untersuchungsmethoden* appeared in the same year, 1897, and this went through numerous editions up to Dr. Schmorl's death in the early 30's. Romeis' encyclopedic Taschenbuch flourished over the same period. During the same period the Spanish school of neuroanatomists, led by S. Ramón y Cajal and his pupil, del Río-Hortega, made its extensive contributions to the uses of silver salts in study of the morphology of glia and reticulum. I should not neglect to note the contributions of the zoologist, A. Bolles-Lee, and the cytologists McClung, Cowdry, and Bensley, among others.

At first the efforts of the technologists were aimed largely at making more plainly evident the already known morphologic details of tissue, at facilitating their preparation in optically practical layers to permit microscopic study, at the preservation of grosser spatial relationships in at least relatively unaltered form, at the preservation of morphologic details against the natural process of autolysis.

Much was learned by the early histologists and pathologists from teasing apart small fragments of organs in serum or salt solution and studying the material unstained under the microscope. From this to the application of reagents and dyestuffs to the surviving tissue was a short step. And this procedure has led, among others, to the supravital technics widely used for the demonstration of such substances as mitochondria and "neutral red granules". Still more recently, phase microscopy, whose physical explanation I will not attempt at the moment, has been applied with profit to surviving material of this type, and owing to differences in refractive indices, has permitted differentiation of nuclei and various cytoplasmic structures in the living cell.

Ultraviolet micrography at selected wavelengths has yielded interesting morphologic details which depend on differences in the absorption spectrums of various chemical cell components. For instance, thymonucleic acid absorbs radiant energy rather strongly at about 270 millimicrons ($\mu\mu$). These absorption characteristics have been used for the localization of specific substances, using radiant energy of specified wavelengths for photography. The process is cumbersome, requires quartz optics for use of short wave (200–300 $\mu\mu$) ultraviolet and requires photographic recording for study.

Of much broader application is the more recently introduced technic of ultraviolet fluorescence microscopy. This depends on the property possessed by many natural substances of generating light of longer wavelength when "excited" by short wave visible or ultraviolet light. Much work has been done in recent years, utilizing the 365 $\mu\mu$ mercury arc line as a light source and inserting ultraviolet absorbing filters in the light path between the object and the eye of the observer. This technic permits direct visual observations and, hence, allows examination of wide areas of a given object. The main value of fluorescence microscopy would appear to be in detection of the natural fluorescences of various tissue components, such as vitamin A, lipofuscins, lipoids, amyloid, ceroid, riboflavin, chlorophyll and atabrine.

The practice of using fluorescent dyes for the staining of nonfluorescent objects, to my mind, presents no special advantage over the use of nonfluorescent dyes and

visible light. The principles of staining with dyestuffs depend on the chemical characteristics of the dye and not on its color *per se* and, hence, fluorochromes will follow the same general rules of staining as other acid and basic dyes.

The auramine technic for demonstration of acid-fast bacilli by fluorescence microscopy permits *detection* of organisms at only slightly lower magnifications than lightly counterstained Ziehl-Neelsen preparations. For *identification* higher magnifications are still necessary, and in tissue work the method has the disadvantage of suppressing entirely tissue details. For fluorescence microscopy fixed tissues may be used, and paraffin and frozen sections may be employed when due regard is taken for the specific behavior toward fixatives and toward dehydrating agents and solvents of the substances to be studied.

Returning to the thesis on methods of preparation, most histopathology is done with section methods on fixed tissue. The practice of fixation was introduced first to preserve tissue against autolytic digestion, and second to harden it to a consistency which would permit preparation of sections. Since sectioning was first done free-hand with a razor on unimbedded tissue, this requirement of hardening was of much greater importance than it has become since the introduction of freezing and imbedding microtomy. When the feature of hardening can be neglected, fixation methods should be selected on the basis of adequacy of preservation of the special tissue elements to be studied. For the demonstration of enzymes, low-temperature fixation in acetone or alcohol is preferred. This has been applied particularly to the demonstration of phosphatases and lipase, but we have found it applicable also to dopa-melanase. Peroxidases appear to be more resistant, and for hemoglobin, hemolytic fixatives must be avoided. In this instance, the primary requirement seems to be that the proteins of the enzyme escape the denaturation incident to other fixing methods. The same requirement applies also to ribonucleic acid and certain other substances of related nature, when application of enzyme-digestion tests for their identification is desired. These are much more easily digested by the specific enzymes after brief cold fixation with alcoholic fixatives than after longer room-temperature fixations with aqueous fixatives.

Of much interest in this connection is the quick freezing and *in vacuo* desiccation technic. This method preserves protein substances, including enzymes, in even less altered form than the cold acetone technic now preferred by Gomori for enzyme work. In this method, tissues are quickly frozen at very low temperatures, which keeps the ice crystals very small and thereby prevents the distortion produced by ice on slow freezing. *In vacuo* desiccation, such as is used in lyophilizing serums, is followed directly by paraffin infiltration. This may be abbreviated to a matter of ten or fifteen minutes by use of vacuum to remove air, or in the case of cold acetone fixation, the volatile paraffin solvent employed. The purpose of abbreviation is again to avoid protein denaturation by prolonged heating. Some enzymes are destroyed by moist heat between 55 and 60 C.

The freezing and desiccation apparatus is, unfortunately, cumbersome and not well adapted to routine use.

It is perhaps interesting at this point to note that the protein chemists have

been using various water-alcohol mixtures at temperatures in the neighborhood of -30°C . for fractionation of proteins without denaturation, and it may well be that we can transfer these technics to histology when we wish to study enzyme localization, protein digestibilities and so forth.

For the demonstration of fatty substances, fixation and preparation methods which do not remove these substances are to be selected. Neutral fats require avoidance of fat solvents throughout fixation, sectioning, staining and mounting procedures. Cholesterol and its esters exhibit similar properties. Phosphatides are rendered insoluble by formaldehyde, especially in the presence of calcium salts, and may then be stained with a myelin type of hematoxylin stain, after Smith-Dietrich, or with oil-soluble dyes, even after paraffin imbedding. The latter property is that of the Ciaccio-positive lipoids demonstrated with oil-soluble dyes which, like myelin, require formalin fixation and chromation before paraffin-imbedding. Still other lipoids are insoluble even in the unfixed state and may be demonstrated even in Carnoy-fixed material with myelin-type stains or with oil-soluble dyes. Ceroid and oxidized unsaturated fats, such as linolein and morrhuin, may be thus shown.

Formalin is also a good protein coagulant and gives quite useful pictures for routine diagnostic work. Heavy metals, picric acid and chromates give harder fixations with less cytoplasmic shrinkage. Acetic acid in various fixatives tends to dissolve various oxyphilic granules, such as zymogen granules.

Sectioning procedures are based on frozen material, either fixed or unfixed, and on material infiltrated with water-soluble imbedding mediums, such as gelatin, agar or certain soaps, on material dehydrated and infiltrated with various nitrocelluloses, celloidin being the traditional one, the low viscosity products being more rapid and giving firmer consistency; and on material dehydrated, cleared and infiltrated with various waxy materials, of which paraffin is the most widely used.

While various other dehydrating agents have enjoyed a considerable vogue, such as acetone, dioxane, isopropanol, *n*-butanol, the traditional ethyl alcohol is still the most generally satisfactory and cheapest, if it can be obtained free of tax. Of paraffin solvents, there are a considerable number which dissolve variable amounts of paraffin at room temperature, but which are all freely miscible at paraffin-oven temperatures. Benzene, chloroform, dioxane, toluene, carbon disulfide, give rise to toxic vapors in concentrations which may be sufficient to induce symptoms in laboratory workers. The less volatile carbon tetrachloride, xylene, and the less toxic petroleum ethers, including lead-free 100 octane gasoline, are less objectionable on this score.

The introduction of mechanical devices for continued gentle agitation and periodic change from one fluid to another, ending in paraffin or in a paraffin solvent, have enabled the routine use of technics requiring as little as eighteen to twenty-four hours from the operating room to the paraffin block ready for the microtome. This largely overcomes the advantage of the quick frozen section procedure for all but emergency diagnosis. And it is precisely in that circumstance, when the diagnosis is dubious on gross examination of the surgical

specimen, that the frozen section technic is least likely to give a satisfactory picture for diagnosis and the heat technic in a paraffin section is necessary to enable one to furnish the answer to the ever urgent question, "Is it carcinoma or not?"

Also, in decalcification of bone, mechanical agitation has proved valuable. Continuous gentle agitation reduced by about one-half, the time required for decalcification in 5 per cent formic acid. This fluid has been found to decalcify nearly as rapidly as 5 per cent nitric acid, and in distinction from the latter allows use of azure eosin stains, such as Giemsa's stain. Sulfurous acid (H_2SO_3) can be used, but in this instance exposure should be less than forty-eight hours. Formic acid decalcification can be prolonged for days without damage to subsequent staining of marrow cells.

Staining procedures applicable to prepared frozen or imbedded section have multiplied from the simple single nucleocytoplasmic stains of the 60's and 70's, the nuclear and cytoplasmic stains such as hematoxylin-eosin which are still widely used, to methods for connective tissue fibrils: collagen, elastin, reticulin, glia; for fats, fatty acids, cholesterol, phosphatides, myelins, lipofuscins; for polysaccharides and glycoproteins including glycogen, starch, various mucins, cartilage, amyloid, hyalin, and other related or similar substances; for vitamin A, C, riboflavin, carotene; for various metals; for radioactive substances; for the pigments; and for the enzymes, such as phosphatases, peroxidases and lipase. And we momentarily expect others. Further, a great deal of chemical and histochemical information is now available as to the nature of many long-recognized morphologic entities.

Among the more spectacular of the recent methods are the various applications of the Schiff aldehyde test with sulfur dioxide leucofuchsin to fixed tissues after varying preliminary treatments. Treatment of frozen sections with mercuric chloride after oxidation in air or briefly with a little hydrogen peroxide (Leblond) before the leucofuchsin gives the colamine glycerophosphate acetals known as plasmalogens. Hydrolysis with hydrochloric acid gives thymonucleic or desoxyribonucleic acid, the characteristic protein of metazoan nuclei. Oxidation with fresh potassium permanganate solution gives glycogen and certain of the epithelial mucins. Using chromium tri-oxide as oxidant, most of the epithelial mucins as well as glycogen, certain colloid substances and yeast and mold chitins, are rendered Schiff-positive. If the oxidant is periodic acid (HIO_4), the Schiff-positive substances include glycogen, epithelial mucins, collagen, reticulin and basement membranes, thyroid and hypophyseal colloid, salivary zymogen granules, Paneth cell zymogen granules, yeast, mold and certain bacterial capsules, cerebral and prostatic corpora amylacea, certain pigment and prepigment granules, chromaffin and certain granules in macrophage-like cells. Collagen and Paneth granules may be taken from red-purple to orange by subsequent staining with picric acid. Liang's method for axones employed brief fixation, one hour in 1 per cent acetic or formic acid followed by a sulfur dioxide rinse, and then one to three hours' treatment with leucofuchsin.

The use of enzyme treatment of tissues to remove specified elements dates

back to Mall's isolation of organ reticulum by "pancreatin" digestion in the 90's. Salivary digestion to remove glycogen has been used for many years, and recently the purified enzymes ptyalin, amylopsin and malt diastase have been used for the same purpose. A purified ribonuclease has been used for the identification of ribonucleic acid in tissues. This enzyme, ribonuclease, removes the basophilic component of the cytoplasm in pancreatic acini, lymphocytes, plasma cells, various epithelia, salivary acini, gastric chief cells, and the tigroid substance of nerve cells. It does not affect mucus, cartilage, mast cell granules, thymonucleic acid. Hyaluronidase from beef testis has been employed in the differentiation of the hyaluronic acid connective tissue mucins from the chondromucins and epithelial mucins. It would seem that a considerable expansion of this field could be made, bearing in mind the previously recited influence of denaturant fixatives on the digestibility of protein substances. It is noteworthy that the fixation method has no apparent influence on the digestibility of glycogen by amylolytic enzymes.

Recent work has shown the essential basic similarity of the picric acid, phosphomolybdic acid, and other acid methods with such dyes as anilin blue, acid fuchsin, light green and others for demonstration of collagen and reticulum. In fact, buffering mixtures of the Mann type, containing methyl blue or anilin blue and eosin to a fairly acid level, 3.5 to 3.8, renders them quite selective stains for connective tissue as well as for eosinophil and cyanophil cells in the hypophysis.

Stains of this type tend to stress the similarities of collagen and reticulum, while the tryptic digestion method, the ammoniacal silver reduction method and the periodic (HIO_4) acid-Schiff with picric acid counterstain stress the differences. Mallory based his conclusion of identity on long experience with phosphomolybdic acid anilin blue stains. Foot took the contrary conclusion from work with trypsin and with silver. The new periodic acid-Schiff evidence supports Foot's and Mall's views.

There has been a certain amount of renewed interest in microincineration of late years. This procedure is performed by heating sections in a properly designed furnace to about 500 C. Some prefer to heat first in nitrogen and then admit oxygen or air at 500 to 600 C. By this procedure the mineral elements are left. If the freezing and drying technic is used, even such substances as soluble sodium and potassium salts are left. Identification is difficult, but certain chemical tests may be applied to the spodiograms by previous collodion spraying to immobilize the ash.

In the practice of autoradiography for localization of radioactive tracer substances, a film is placed in contact with unstained or stained sections and exposed long enough to produce an image. With alpha emitters the procedure of floating sections onto film or of pouring emulsion onto unstained sections would seem to give the best resolution. With beta emitters, the contact, the poured emulsion and strip film procedures are used. Gamma emitters give poorer resolution than beta, but the same procedures are used.

Localization is much better in those preparations in which the stained section and film are coherent than in the separate preparations, but histologic staining is

difficult. Gelatin has a great avidity for stains. Pre-staining has been tried, but the photographic process tends to remove the stains.

In conclusion, I will touch briefly on the currently popular smear methods of diagnosis for cancer. The older ones among you will recall that we have had a number of similar episodes of popularity of smear methods for cancer diagnosis, which lapsed into disuse in the intervals. I can recall searching pleural exudate smears for cell clumps suggesting cancer cells in 1919. Cytologic diagnosis was as difficult then as now, although it has had prominent advocates (MacCarty, Foot and others) before Papanicolaou, and it disregards the primary criterion on which my teacher, Ophüls, used to insist, that the tumor cells break through the basement membrane and thus exhibit the essential characteristic of "invasiveness". The Papanicolaou stain is essentially a modification of Mallory's anilin blue stain, such as those used by the biologists in the study of oestrus cycle of rodents on vaginal smears. The stain itself, as Papanicolaou states, is not specific for malignant cells, and many other staining methods are being used.

CONSTANT OLIGOSPERMIA AND PERIODIC OLIGOSPERMIA*

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In evaluating male fertility by semen analysis, the only quality which is reliable and objectively determinable is the number of spermatozoa.^{3, 6, 8} A reduction in the number of spermatozoa is usually accompanied by a reduction in their motility and in the percentage of morphologically normal spermatozoa.^{6, 7} Reported spermatoc counts compatible with fertility vary between 60 and 120 million per cc.^{3, 4, 10} We have, however, observed a number of men with spermatoc counts of less than 10 million per cc. whose wives, nevertheless, became pregnant. Extraconjugal relations were excluded in these women in view of their extremely orthodox religious outlook. In a much larger group of men with similarly low spermatoc counts, we have observed infertility. These observations led us to the assumption that two types of oligospermia may exist. In one the spermatoc count is constantly low, while in the other, variations in spermatogenesis may occur. Thus, in this second postulated type of oligospermia, fertilization might take place at a time when higher spermatoc levels were reached. In order to verify this hypothesis we performed repeated semen analyses on a group of men with oligospermia.

We examined a selected series of 30 men whom we had considered responsible for barrenness, on the basis of the first sperm examination. The first spermatoc count in these cases was always below 30 million per cc. and varied between 1 and 10 million per cc. in 15 men, between 10 and 20 million per cc. in 10 men and between 20 and 30 million per cc. in 5 men. The number of spermatozoa in the ejaculate in all cases was below 90,000,000 per cc. (oligospermia), and sperm motility, as well as the number of morphologically normal forms, was correspondingly low.

METHODS

In all of the 30 cases of male sterility studied, the wives were excluded as responsible for barrenness. No vaginal, cervical or uterine conditions were observed which might be expected to interfere with fertilization. Patency and normal function of the Fallopian tubes, as well as ovulation, were confirmed by kymographic utero-tubal insufflation and salpingography, and by the finding of a pregestational endometrium on histologic examination.

Semen analyses were performed after routine general and genital examination of the patients. The subjects selected for this study had no organic disease, chronic intoxication or avitaminosis. No important changes occurred during the period of study in the patients' dietary habits or environment.

* Received for publication, April 19, 1948.

Specimens were collected in a dry, graduated, glass container after masturbation or coitus interruptus, following a four-day period of abstinence from sexual indulgence. The semen was examined thirty to sixty minutes after ejaculation. The volume, color, viscosity and the rate of mucolysis were noted.^{10, 11} The spermatozoa were counted by the pipet dilution method, using a saturated solution of sodium bicarbonate containing 1 per cent phenol as diluent.⁵ Counts were made in a Thoma counting chamber, sperms were examined for motility two, twelve and twenty-four hours after the ejaculation,² and sperm morphology was studied in smears stained with hematoxylin-eosin.

TABLE 1
VARIATIONS IN COUNTS, MOTILITY AND MORPHOLOGY OF HUMAN SPERMATOZOA

TYPE OF SPERMATOGENESIS	NO. OF PATIENTS	VARIATIONS IN SPERMATOZOA COUNT	MAXIMAL RANGE IN SPERMATOZOA COUNT IN ANY TWO SPECIMENS FROM THE SAME PATIENT	MOTILE SPERMATOZOA, TWO HOURS AFTER EJACULATION	PATHOLOGIC FORMS
		<i>per cc.</i>	<i>per cc.</i>	<i>per cent</i>	<i>per cent</i>
Normal		60,000,000 to 120,000,000 ^{1, 10}		75 ¹¹	20 ⁹
Periodic oligospermia	9	2,500,000 to 69,000,000*	43,500,000	20-75	10-35
Constant oligospermia	21	800,000 to 31,000,000**	<12,000,000	0-35†	25-62‡

* In one case of periodic oligospermia, a spermatoc density of 130 millions per cc. was observed. This exceptionally high value is not included in this table.

** It should be noted that, although the range in the spermatoc density for the entire group is over 30 million per cc., the maximal range in any one individual never exceeded 12 million per cc.

† In two patients motility ranged between 40 and 60 per cent.

‡ In one patient 8 to 26 per cent of pathologic forms were observed on serial semen analyses, and in another patient, 12 to 22 per cent.

Five to seven semen analyses were made in each case during the period of observation which lasted from four to twenty-three months. No treatment was administered during this period.

RESULTS

On the basis of the repeated spermatoc counts in this series, we were able to classify our patients in two distinct groups with respect to their spermatogenic activity.

Group 1. In this group comprising 21 out of the 30 patients, the spermatoc densities ranged between 800,000 and 20,000,000 per cc. on first examination. On serial examination, spermatoc densities reached a maximal level of 31,500,000 per cc. in two instances. In none of the patients in this group did the difference between any two spermatozoal counts exceed 12,000,000 per cc. (Table 1). The other seminal characteristics associated with spermatogenesis (sperm

motility and the percentage of pathologic forms) also remained fairly constant. Sperm motility was always low and, in all but two patients, varied between no motile spermatozoa and 35 per cent motility. The number of pathologic forms varied between 25 and 62 per cent except in two instances in which 8 per cent and 12 per cent of pathologic forms were found. No pregnancies occurred in this

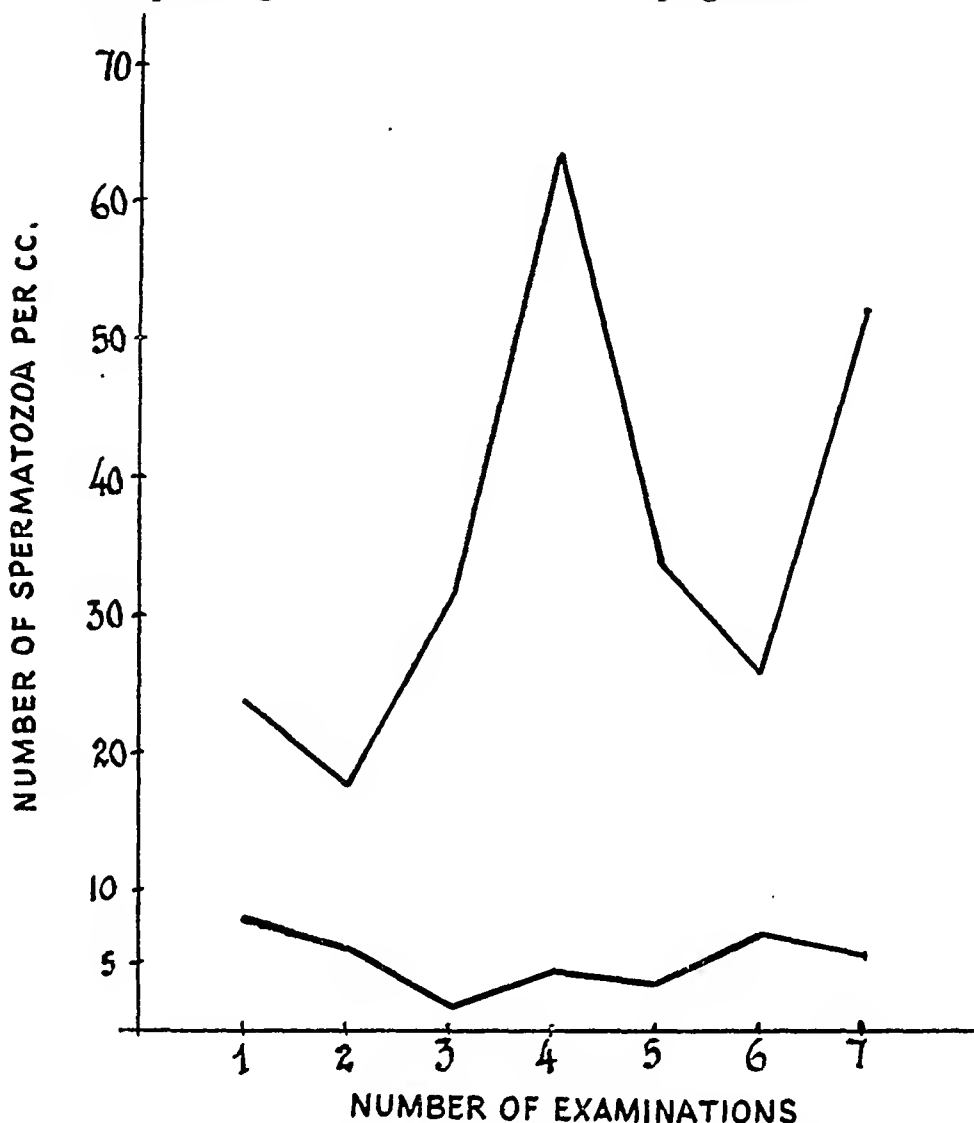


FIG. 1. Upper curve, periodic oligospermia. R.T., aged 40, married 7 years; 4 years' secondary sterility; sperm counts characterized by periodic variations. Lower curve, constant oligospermia. M. L., aged 32, married 5 years, primary sterility; constancy of low spermatoc count on repeated examinations. N. B.: The indicated numbers of spermatozoa per cc. represent millions.

series during the period of observation. The constant occurrence of abnormal spermatogenesis during successive examinations in this group of patients was, therefore, called *constant oligospermia* (Fig. 1).

Group 2. This group, consisting of 9 patients, differed from the preceding group in that the spermatoc characteristics observed in successive specimens varied strikingly. In all the patients in this series, initial examinations yielded values ranging between 2,500,000 and 30,000,000 per cc. In subsequent

examinations, however, one or more specimens yielded values higher than 46,000,000 per cc. in 4 men and higher than 60,000,000 per cc. in 5 men. Observed differences in sperm density on repeated semen analyses of the same patient were extremely variable, reaching 43,500,000 per cc. In a number of instances we observed that, whereas the second and in some cases the third examination revealed no significant variation from the first, the number of spermatozoa increased up to normal levels in the fourth or fifth examinations. The motility and the percentage of pathologic forms in this group also varied, but to a considerably less degree than did the spermiatic density. The percentage of pathologic forms ranged between 10 and 35. Sperm motility in this series varied between 20 and 75 per cent. In 5 patients, despite rises in the number of spermatozoa, the percentage of pathologic forms showed no significant variation, whereas in the others a rise in the number of spermatozoa was accompanied by a corresponding rise in the percentage of normal forms with improved motility. This type of oligospermia, characterized by striking periodic variations in spermatogenetic activity, was called *periodic oligospermia* (Fig. 1). Conception occurred during the period of observation in two of these 9 hitherto barren marriages. It seems very probable that a number of men considered "normal" or fertile on the basis of one sperm examination might present oligospermic values at subsequent examinations and would, therefore, be included in this group.

DISCUSSION

Two types of oligospermia were distinguishable only after several examinations were performed. The importance of repeated sperm examination in the evaluation of male fertility is often overlooked or underestimated, because the spermiatic picture is generally considered to be a relatively constant condition,^{1, 8, 11} similar to the erythrocyte count. Our patients demonstrated that the regulating mechanism of spermatogenesis cannot be compared to that of hematopoiesis. We have observed that at least five semen analyses, each taken after four days' abstinence from coitus, should be performed before a definite conclusion can be drawn as to spermatogenetic function.

In the light of our observations, it is clear how pregnancy may occur in certain instances with very low spermiatic counts, since spermatozoa counts in periodic oligospermia occasionally rise to levels usually considered sufficiently high to induce conception.

The observed wide variations in the spermiatic counts of certain men on repeated examination (periodic oligospermia) leads one to be skeptical in the evaluation of various therapeutic agents recommended and employed against oligospermia, since spontaneous variations in spermatogenesis might be misinterpreted as due to therapy when such drugs are administered. Therapeutic results in oligospermia should be assessed only in cases with constant spermatogenesis, i.e., in men who exhibit low spermiatic densities on at least 5 consecutive examinations. The occurrence of spontaneous variations in spermatogenetic function in oligospermic men may also be of importance in cases of disputed paternity met with in forensic medicine, since we have observed that a man with

a very low spermatoc count may still be fertile if he is subsequently found to exhibit the periodic type of oligospermia.

From the foregoing it is evident that an unfavorable prognosis should not be made in a case of oligospermia until it is definitely established, after repeated examinations, that no great variations occur in the spermatogenetic function.

SUMMARY

1. In a study by means of repeated semen analyses of 30 cases of male infertility, two types of oligospermia were distinguished:

a. *Constant oligospermia* (21 cases), characterized by a constantly low spermatoc density on five or more examinations. In this group no pregnancies occurred during the period of observation.

b. *Periodic oligospermia* (9 cases), characterized by striking periodic variations in spermatogenesis with normal spermatoc densities encountered in one or more of the serial examinations. In this group, fertilization took place in 2 of 9 women during the period of observation.

2. We have stressed the importance of serial sperm examination as a means of determining the type of oligospermia and the possibility of conception, and the possible value of sperm analysis in forensic medicine.

3. Therapeutic studies in oligospermia should be performed only in cases with constant oligospermia. Many apparently successful results in the treatment of oligospermia may be ascribed to the spontaneously occurring increases in spermatogenesis observed in "periodic oligospermia".

4. Diagnosis of male sterility should not be made unless five examinations, made after periods of abstinence from sexual indulgence of at least four days, have all shown low spermatoc counts. These five examinations should be performed during a period of two months or more.

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INCREASED BLOOD PLATELET CLUMPING IN THROMBOEMBOLIC DISEASE*

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It was the purpose of this study to evolve, if possible, a simple standard method of designating the degree of clumping in blood platelets for clinical and hematology use. This communication reports the observations made, as a result of a study of conventional stained blood films, on the degree of clumping of platelets in healthy persons and in patients with thromboembolic disease.

METHOD AND RESULTS

Ordinary routine differential blood films were made on glass slides, stained with Wright's solution and observed under the oil immersion lens. The presence of blood platelet clumps and the number of platelets per clump were noted and recorded. Clumping was considered normal if the blood film did not reveal more than 10 platelets to the clump (Fig. 1), as increased or abnormal if there were more than 10 platelets to the clump (Fig. 2). Clumps containing more than 15 platelets not infrequently presented a syncytial arrangement with an almost transparent hyaline matrix in which platelets of varying sizes and shapes were embedded (Fig. 3).

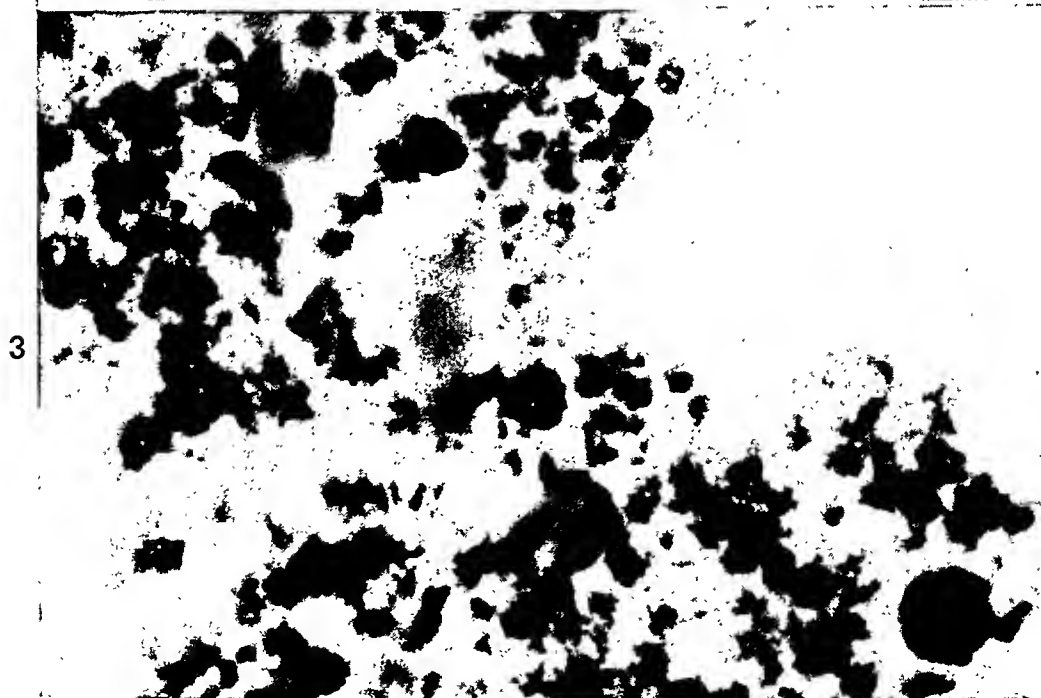
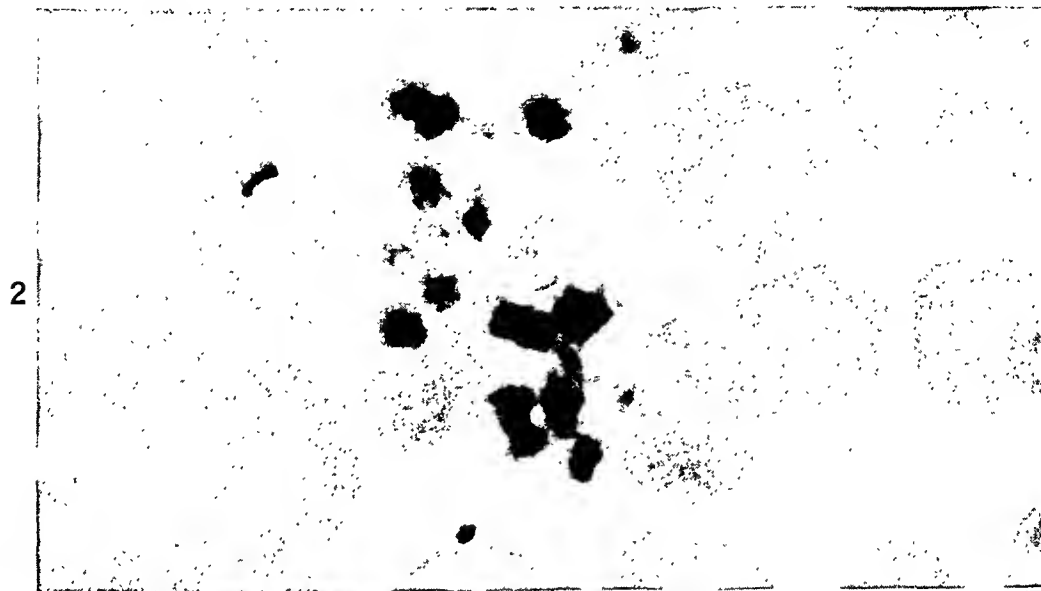
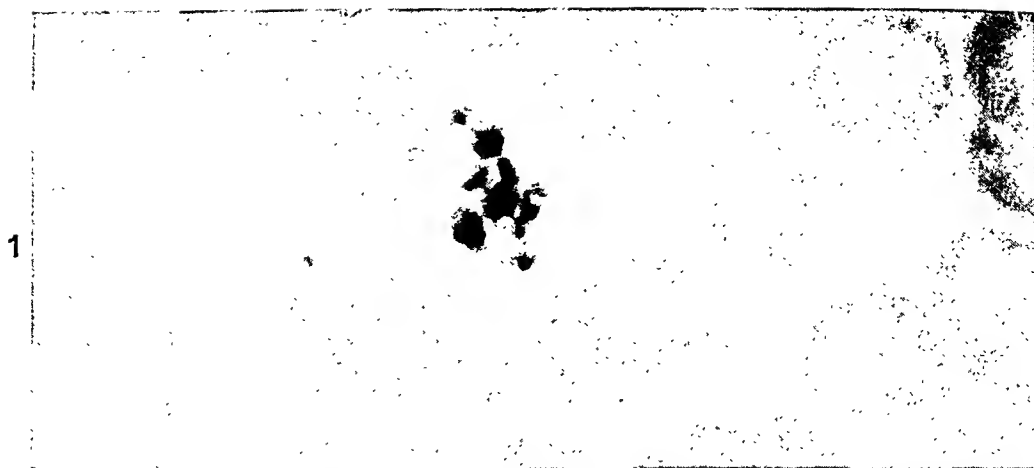
In 100 presumably normal control subjects (Table 1) without detectable clinical disease, clumping was normal in 92 per cent and designated as increased or abnormal in only 8 per cent. Of 100 patients with various types of thromboembolic disease, clumping was of normal type in 19 per cent and designated as increased in 81 per cent.

DISCUSSION

It was considered that variation in thickness of the film might affect the size and number of clumps noted. Accordingly, thin and thick films were prepared, but there was no appreciable change in the clumping picture. When the films were prepared so that the cellular elements were mostly displaced to one end so as to concentrate all the cellular elements including the platelets, there was also no significant alteration in the clumping pattern. The question arose whether this pattern persists in the individual case. Thus far, rechecks on many of these patients have revealed no significant change; in a few instances there were slight deviations which were ascribed to complicating factors such as a cold or fever.

In an effort to determine the factor or factors responsible for variation in the degree of clumping, we tried to ascertain whether there was a causal relationship

* Received for publication, April 17, 1948.



FIGURES 1—3.

between increased platelet clumping and (1) thrombocytosis and megakaryocytosis, (2) increased erythrocyte sedimentation rate, (3) leukocytosis, and (4) fever.

It became apparent early in our study that increase in number of platelets might possibly play some role in the clumping phenomenon. Accordingly, we analyzed this relationship, as shown in Table 2. In the citrate diluent method, which we used for counting platelets, the normal value ranges from 200,000 to 400,000 platelets per cu. mm. From a review of our data it may be stated that while thrombocytosis is usually associated with an increased tendency to clumping of platelets, such a tendency is not necessarily associated with, or caused by, thrombocytosis.

A comparative study of sternal bone marrow and blood platelet clumping was carried out in 51 consecutive unselected patients. This collateral study disclosed that megakaryocytosis apparently parallels thrombocytosis in its relationship to clumping tendency.

In 29 patients (Table 3) both blood platelet clumping studies and erythrocyte sedimentation rate determinations were made to determine if any parallelism existed between clumping of platelets and the rate of sedimentation of red cells. From the data it may be inferred that an increased sedimentation rate of erythrocytes is generally to be expected in patients whose platelets show an increased tendency to clumping.

White blood cell and differential counts were made from 48 patients with thromboembolic disease (Table 3), who were also studied for degree of blood platelet clumping. This study shows that while leukocytosis and increased clumping frequently co-exist, the relationship is not causal but coincidental.

Of the 51 patients (Table 3) with available temperature charts, 23 manifested a febrile reaction. Analysis of the data indicates no apparent etiologic influence of fever on the clumping tendency.

The actual number of platelets seems to govern the extent of the clumping. While clumping is frequently associated with leukocytosis and/or increased sedimentation rate and sometimes with fever, this correlation is not consistent. It is noteworthy that 81 out of 100 patients with thromboembolic disease exhibited increased clumping. By contrast only 8 out of 100 apparently normal persons showed increased clumping. The assumption seems justified, therefore, that a tendency to increased clumping often reflects a tendency to thromboembolic disease.

FIG. 1. The clumping is of normal distribution. One clump of platelets and two discrete platelets in an apparently healthy subject.

FIG. 2. The clumping is moderately increased (1 plus). A large clump of about 15 platelets of the loosely packed variety and one small clump of 5 platelets in an apparently healthy subject ("thrombophilic").

FIG. 3. The clumping is markedly increased (2 plus). Huge clump containing innumerable platelets was found in a patient with thromboembolic disease. Note the synetial arrangement. With Wright's stain the cluster presents a sky-blue almost transparent hyaline matrix in which platelets of varying sizes and shapes are embedded.

It is of interest that 8 subjects apparently free of disease evidenced a tendency to increased clumping of platelets. It is possible that these individuals may

TABLE 1
BLOOD PLATELET CLUMPING IN HEALTH AND THROMBOEMBOLIC DISEASE

DIAGNOSIS	NO. OF PATIENTS	TYPE OF BLOOD PLATELET CLUMPING		
		Normal*	Increased†	
			1 Plus	2 Plus
A. Apparently healthy control subjects.....	100	92	7	1
B. Patients with thromboembolic disease.....				
1. Venous thromboembolism.....	33	6	18	9
2. Peripheral vascular disease with gangrene.....	23	2	12	9
3. Arteriosclerosis obliterans.....	19	6	6	7
4. Coronary artery thrombosis.....	12	3	9	
5. Cerebral thrombosis.....	5	0	4	1
6. Postthrombotic varicose ulcer.....	4	1	2	1
7. Arterial embolism.....	3	1	2	
8. Thromboangiitis obliterans.....	1	0		1
Totals.....	100	19	53	28

* Up to 10 platelets per clump.

† 1 plus, 11 to 15 platelets per clump; 2 plus, 16 or more platelets per clump.

TABLE 2

CORRELATION OF DEGREE OF CLUMPING AND COUNTS OF BLOOD PLATELETS IN 115 SUBJECTS

	PLATELET COUNT		CLUMPING	
			Normal	Increased
A. Apparently healthy control subjects	Increased	3	1	2
	Normal	43	41	2
	Decreased	2	2	0
	Total	48	44	4
B. Patients with thromboembolic disease	Increased	27	1	26
	Normal	40	17	23
	Decreased	0	0	0
	Total	67	18	49
Total all cases.....		115	62	53

possess an abnormally labile thrombosing mechanism as a result of which they may be susceptible to thrombosis or embolism, especially when subjected to coagulative stimulants such as venostasis, anesthesia, pregnancy, operative pro-

TABLE 3
CORRELATION OF DEGREE OF CLUMPING OF PLATELETS, SEDIMENTATION RATE,
LEUKOCYTOSIS AND FEVER

CASE NO.	DIAGNOSIS	DEGREE OF CLUMPING	INCREASED SEDI-MENTATION RATE	LEUKOCYTOSIS	FEVER
1	Thrombophlebitis	2 plus	No	Yes	Yes
2	"	2	—	—	No
3	"	2	—	—	No
4	"	2	Yes	Yes	No
5	"	2	No	No	No
6	"	1	—	No	Yes
7	"	1	—	No	Yes
8	"	1	—	Yes	Yes
9	"	1	—	No	No
10	"	1	Yes	Yes	Yes
11	"	1	—	No	Yes
12	"	1	—	Yes	No
13	"	1	Yes	Yes	Yes
14	"	Normal	—	Yes	No
15	"	Normal	No	No	Yes
16	"	Normal	Yes	No	Yes
17	"	Normal	No	No	No
18	Peripheral vascular disease with gangrene	2	Yes	Yes	Yes
19	" "	2	Yes	Yes	No
20	" "	2	—	Yes	Yes
21	" "	2	Yes	Yes	Yes
22	" "	2	—	No	Yes
23	" "	2	Yes	No	No
24	" "	2	No	Yes	No
25	" "	2	Yes	Yes	Yes
26	" "	2	Yes	Yes	No
27	" "	1	—	No	Yes
28	" "	1	—	Yes	Yes
29	" "	1	—	No	Yes
30	" "	1	—	No	No
31	" "	1	—	No	No
32	" "	1	—	No	No
33	" "	Normal	—	No	No
34	" "	Normal	—	No	Yes
35	Arteriosclerosis obliterans	2	Yes	Yes	Yes
36	" "	1	—	—	No
37	" "	Normal	No	No	No
38	Coronary artery thrombosis	1	No	No	No
39	" "	1	No	No	No
40	" "	1	Yes	No	No
41	" "	1	No	Yes	Yes
42	" "	1	No	No	—
43	" "	1	No	No	Yes
44	" "	1	No	No	No
45	" "	Normal	No	No	No
46	Cerebral thrombosis	1	Yes	No	No
47	Postthrombotic varicose ulcer	2	—	No	No
48	Arterial embolism	1	Yes	No	Yes
49	" "	1	No	Yes	Yes
50	" "	Normal	—	No	No
51	Thromboangiitis obliterans	2	No	No	No

cedures, hemorrhage and/or infection. Further study may determine if it is thus possible to identify potential "thrombophiliacs" merely by their blood smear. If so, such persons may then be protected by proper anticoagulation measures during pregnancy and infection, prior to anesthesia and before and after operation.

SUMMARY

A simple method of grading the tendency to clumping of blood platelets has been presented. It was observed that platelets of patients with thromboembolic disease showed a greater tendency to be clumped, or to appear in large clusters, than did the platelets of normal persons. This finding may possibly be of value in indicating a tendency of certain persons to develop thrombosis.

BOOK REVIEWS

Manual of Veterinary Bacteriology. Ed. 3. By RAYMOND A. KELSER, D.V.M., Ph.D., Brigadier General United States Army, Retired; Professor of Bacteriology and Dean of the Faculty School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, and HARRY W. SCHOENING, V.M.D., In Charge, Pathological Division, Bureau of Animal Industry, U. S. Department of Agriculture, Washington, D. C. 767 pp., 101 figs., 12 tables. \$6.50. Baltimore: The Williams and Wilkins Company, 1948.

It is not often that one finds a textbook written primarily for the training of students by two scientists who have had such a wide and varied practical experience in the fields of bacteriology, protozoology, virus diseases and immunology. The book is divided into eleven sections. The subject matter is introduced by a thorough and not too wordy discussion of bacteria, their morphology, physiology and classification, followed by bacteriologic methods. The section dealing with immunity is weakest in that stress is given to the older conceptions of this subject and little, if any, consideration to those that have been advanced in recent years. Those sections devoted to pathogenic organisms, viruses and protozoa are complete and thorough. The student, practitioner, or research worker will find in the last two sections complete and usable descriptions of all the serologic methods and hematologic techniques that are now being used in the diagnosis and study of animal diseases. Special consideration has been given to methods of preparing many veterinary biologic products and to standard methods for the examination of milk and water. References may be found on many pages for supplementary reading.

This book, although prepared primarily as a student textbook, should find wide use in both veterinary and human medicine.

East Lansing, Michigan

I. FOREST HUDDLESON

The Acute Bacterial Diseases. Their Diagnosis and Treatment. By HARRY F. DOWLING, M.D. Clinical Professor of Medicine, George Washington University. With the collaboration of LEWIS K. SWEET, M.D., Chief Medical Officer in Pediatrics and Infectious Diseases, Gallinger Municipal Hospital, and HAROLD L. HIRSH, M.D., Assistant Professor of Medicine, Georgetown University. 465 pp., 55 figs., 53 tables. \$6.50. Philadelphia and London: W. B. Saunders Company, 1948.

In the construction of this book on infectious and communicable diseases, the author has employed a new approach. The diseases are identified and discussed according to their etiologic agents. The book is divided into four large divisions, Part One dealing with the diagnosis and treatment of acute bacterial diseases, Part Two including diseases caused by cocci, Part Three diseases caused by bacilli, and Part Four being a discussion of the bacterial diseases in which exotoxins are a major factor.

As the author states in the preface, "Details of epidemiology and pathology have not been included in every instance." The book is apparently not intended for use by the clinical pathologist but is of inestimable value to the practicing physician. The orderliness and clarity of presentation makes for ease of reading and aids in rapid reference. The appendix includes rapid procedures for the determination of sulfanilamide in blood and urine, penicillin assay, and streptomycin assay.

Detroit

MARK DALE

Medical Writing. The Technic and the Art. Ed. 2. By MORRIS FISHBEIN, M.D., Editor of the Journal of the American Medical Association, with the assistance of JEWEL F. WHELAN, Assistant to the Editor. 292 pp., 36 illus. \$4.00. Philadelphia and Toronto: The Blakiston Company, 1948.

The second edition of this handbook, published ten years after the first edition, contains

an additional 80 pages and numerous added details and refinements in presentation. A chapter on Indexing has been included. The typography is more pleasing in appearance and easier to read. For all who are concerned with medical writing, this book is invaluable.

Many of the numerous individual variations in style adopted by most medical periodicals are illogical and confusing. Thus, each journal has its own rules regarding spelling, abbreviations, punctuation, typography, format of tables and illustrations, arrangement of references in the text, and bibliographic listing. While the adoption of some special features may possibly lend distinctiveness to a particular journal, for most details of style, it would certainly be advantageous to authors if medical journals would adopt a standard code. The rules and practices set forth in *Medical Writing* are followed by all periodicals published by the American Medical Association. This volume might well serve as a basis for the formulation of a standard code for medical editors and writers.

Eloise, Michigan

S. E. GOULD

The Care and Management of Laboratory Animals. Handbook of the Universities Federation for Animal Welfare. With an Appendix on Statistical Analysis. Edited by ALASTAIR N. WORDEN, M.A., B.Sc. Milford Professor and Director of Research in Animal Health, University of Wales, Aberystwyth. With a Foreword by Professor T. DALLING, M.A., Director, Veterinary Laboratory, Ministry of Agriculture and Fisheries, Weybridge, Surrey. 368 pp., 70 figs. \$8.50. Baltimore: The Williams and Wilkins Company, 1947.

This handbook, intended as a practical guide for research worker and technicians, has been compiled by a number of expertly qualified workers selected by the Universities Federation for Animal Welfare.

The first chapter deals with the rights of laboratory animals; the concept that laboratory animals have certain rights is accepted as a basic policy of the Federation. A large number of species of animals are treated, particularly with reference to their accommodation, nutrition, breeding, handling and marking, cleaning, parasites and diseases. Included among the species that are the subject of a full discussion are the rabbit, guinea pig, Norway rat, black rat, mouse, deer mouse, cotton rat, field vole, golden hamster, ferret, hedge hog, pigeon, canary, frogs and toads including the toad, *Xenopus laevis*, and freshwater fish. There are also notes on dogs and cats, horses, poultry and reptiles. An appendix of 30 pages is given to statistical analysis.

The book is well written, contains a mass of useful and well-documented data and good bibliographies. It can be recommended without reservation to all who are concerned with laboratory animals. It is well worth having in the laboratory library.

Biology of Disease. By ELI MOSCHCOWITZ, M.D., Physician, Mt. Sinai Hospital, New York; formerly Assistant Professor, Clinical Medicine, Columbia University. 221 pp., 2 charts, 6 tables. \$4.50. New York: Grune and Stratton, Inc., 1948.

The writer has prepared 24 chapters on various medical subjects, on most of which he has previously made original contributions. In these chapters he expresses his concepts of the etiology, pathogenesis and evolution of certain disease states. Some of the subjects treated include pulmonary hypertension, systemic hypertension, arteriosclerosis, periarteritis nodosa, Libman-Sacks disease, polycythemia vera, leukemia, Grave's disease, portal cirrhosis, glomerulonephritis, obesity, peptic ulcer, the sprue syndrome, nephrosis and the hyperkinetic diseases. These make interesting reading. They may be said to represent the ideas and in some instances the philosophy of disease of a scientific medical man during a life-time of observation and writing. For sober students of medicine, these chapters will have considerable interest and will furnish much food for thought.

TECHNICAL SECTION

QUANTITATIVE METHOD FOR DETERMINATION OF UROBILINOGEN IN STOOL AND OF UROBILINOGEN AND BILIRUBIN IN URINE*

HUGH G. BRERETON, M.D., AND S. P. LUCIA, M.D.

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In the course of a study of hemoglobin derivatives, the need for a simple and accurate method for the determination of bile pigments in the urine and stool led to the development of the procedure described in this paper. Our problem was to determine the quantity of urobilinogen aldehyde pigment in these excretions and to exclude photelometrically, substances, chromogenic or otherwise, which might yield a colored product with Ehrlich's reagent. This result could be achieved by selective oxidation of urobilinogen to a nonreactive substance in the control sample, and thus leave the urobilinogen in the test sample free to react with Ehrlich's reagent. The interfering chromogens would, therefore, be common to both samples, and the intensity of the color reaction obtained in the test sample would indicate the direct proportion of free urobilinogen in this sample. With this ideal in mind, a series of oxidizing agents was tried and a final decision was made in favor of a 2.6 per cent solution of sodium hypochlorite. Since sodium hypochlorite oxidizes bilirubin to biliverdin, this reagent may also be used to determine the quantity of bilirubin in a sample of urine. In the latter case, the bilirubin content of the homologous control sample is removed by precipitation with calcium chloride and filtration. Using the techniques described below, we have studied the urinary urobilinogen excretion of 193 subjects, the urinary bilirubin of 132, and the urobilinogen excretion in the stool of 165. In addition, there were 14 subjects in whom all three substances were tested serially five or more times. Based on this experience, we present the following methods.

METHODS

I. Urine

A. *Collection of urine samples.* Twenty-four hour samples of urine are collected in brown glass bottles calibrated to facilitate measurement of the volume output. Five grams of sodium carbonate and 40 ml. of petroleum ether are used as a preservative.⁴ Urine thus treated shows no appreciable loss of urobilinogen. Random samples of urine are not used because of unpredictable fluctuations in the appearance of urinary urobilinogen.

B. *Determination of urobilinogen.* By means of a pipet or separatory funnel, a 40 ml. sample of the urine is obtained free of ether. Five grams of calcium

* Received for publication, July 6, 1948.

chloride is added, the mixture is thoroughly agitated to precipitate the bilirubin and the latter is subsequently extracted by filtration. To a 10 ml. portion of the filtrate, 1 ml. of water and 1 ml. of Ehrlich's reagent (concentrated hydrochloric acid, 75 ml.; distilled water, 75 ml.; p-dimethylaminobenzaldehyde, 10 grams) are added; to another 10 ml. portion are added 1 ml. of 2.6 per cent sodium hypochlorite solution followed by 1 ml. of Ehrlich's reagent. Any precipitate which forms in either sample must be removed by filtration. Any bubbles which arise are dispelled by spinning or tapping the tubes. The tube containing the sodium hypochlorite reagent will serve as the control for the photometric determination of both the urinary urobilinogen and bilirubin, since its urobilinogen content has been oxidized to a nonreactive substance and its bilirubin has been extracted by filtration.

C. *Determination of bilirubin.* The presence of bilirubin in minute amounts in the urine can be detected by the presence of a green to pink spectrum produced by adding a few drops of nitric acid to the filter paper which contains the calcium precipitate of the treated sample of urine. If a color reaction occurs, a 10 ml. portion of the original urine specimen is treated by the addition of 1 ml. of 2.6 per cent sodium hypochlorite solution followed by 1 ml. of Ehrlich's reagent; any bilirubin present is oxidized to the more intensely colored biliverdin and the difference between this and the control sample indicates the quantity of bilirubin present in the urine.

II. Stool

A. *Collection of stool samples.* Random single samples of stool are used because the results obtained compare favorably with those of the usual four-day collection.²

B. *Determination of urobilinogen.* A 5-gram portion of the stool is transferred to the bottom of a 250 ml. Erlenmeyer flask; 140 ml. of water and 5 grams of ferrous sulfate* are added and the mixture is thoroughly stirred by means of an electrical mixer. Ten minutes of agitation usually suffices. Forty ml. of 10 per cent sodium hydroxide is added slowly to the mixture and the stirring process is continued until a homogeneous solution results. The flask is corked and allowed to remain overnight in a dark cabinet in order to insure complete reduction.** Twenty ml. of the mixture is filtered through a #1 filter paper. The test and control solutions are prepared with the following materials added in the order given:

Test solution:	Control solution:
4.5 ml. water	1.0 ml. 2.6 per cent sodium hypochlorite
5.0 ml. stool filtrate	2.0 ml. water
0.5 ml. 5N hydrochloric acid	5.0 ml. stool filtrate
1.0 ml. Ehrlich's reagent	2.0 ml. 5N hydrochloric acid
	1.0 ml. Ehrlich's reagent

* The ferrous sulfate in an alkaline medium reduces the urobilin of the stool to urobilinogen.³

** Naumann¹ recommends three hours at 20 C. or five minutes at 35 C.

The difference in the amount of hydrochloric acid used in the above solutions does not alter the results of the comparison. Less than 2.0 ml. of hydrochloric acid in the control will produce a white cloud upon the addition of Ehrlich's reagent, and more than 0.5 ml. of hydrochloric acid in the test solution will produce oxidation of some of the urobilinogen.

COMMENT

The results of the procedures as applied to the urine will yield the following three test solutions: one containing the urobilinogen aldehyde pigment, one containing bilirubin, and a control containing neither. The natural pigments of the urine and the substances giving a color reaction with Ehrlich's reagent will be common to all three solutions.

Similarly, in the specimen solutions resulting from the procedures done on the stool, the difference in color between the test and the control solutions will be due primarily to the urobilinogen aldehyde pigment in the former. Thus determination of the differences in light transmission* between the above control and test solutions will indirectly give the value of the concentration of urobilinogen and bilirubin in the urine and of urobilinogen in the stool.

CALIBRATION OF THE PHOTELOMETER

A. *Urobilinogen*. A "100 per cent solution" of phenolsulphonphthalein (1 ml. diluted in 1000 ml. water) has been found to give a color comparable to the urobilinogen aldehyde pigment produced from a concentration of 0.721 mg. of urobilinogen per 100 ml. of urine or 100 Gm. of stool.** Serial dilutions of phenolsulphonphthalein are set up and a curve is calibrated for varying concentrations. Similar values on the photelometer indicate comparable values of urobilinogen. In the described procedure, the urine has been diluted in the ratio of 10:12; therefore, the results of the photelometric determination must be multiplied by 1.2 ($1.2 \times \text{concentration of urobilinogen} \times \text{volume of urine in twenty-four hours} = \text{mg. of urobilinogen in twenty-four hours}$). In the stool, a 5-gram sample was mixed with 180 ml. of solution giving a ratio of 1 gram of stool to 36 ml. of solution. The dilution factor in the preparation of the test solution is 2.2, with a final ratio of 1 gram of stool to 79 ml. of solution. Therefore, the photelometric computation of urobilinogen in milligrams per 100 ml. must be multiplied by 79.0 to determine the concentration of urobilinogen in milligrams per 100 grams of stool.

B. *Bilirubin*. A known weight of bilirubin is dissolved in a 5 per cent solution of sodium carbonate. To a series of 10 ml. samples of progressive concentrations of bilirubin, 1 ml. of 2.6 per cent solution of sodium hypochlorite and 1 ml. of Ehrlich's reagent are added. The resulting green color is due to biliverdin and will serve to calibrate the photelometer for the color resulting from a known con-

* Using a Leitz-Mass Photelometer, the #428 filter has been found most selective for the colors involved.

** Watson *et al.*⁶ suggest a Pontocyl Stock Standard as more closely simulating the urobilinogen aldehyde color.

centration of bilirubin. Since the dilutions in this calibration procedure and in the procedure as applied to the urine are the same, no correction is necessary.

CLINICAL OBSERVATIONS

In a study of the urobilinogen excretion of normal subjects, Watson⁵ obtained the following values: in the urine 0.5 to 4.0 mg. per 24 hours; and in the stool, 40 to 280 mg. per day. Using the same technic, Steigmann and Dyniewicz² obtained in normal subjects, the following values for the excretion of urobilinogen: in the urine, 0.2 to 3.0 mg. per 24 hours and in the stool, 30 to 200 mg. per 100 grams. Our own normal values (18 different samples on 7 subjects) for the urinary excretion of urobilinogen were 2.9 to 9.6 mg. with an average of 6.5 mg. per 24 hours, and for urobilinogen excretion in the stools of normal subjects (32 medical students) 38.5 to 226 mg. per 100 grams of stool (one value registered 12.7 mg.)

In order to demonstrate the sensitivity of the test in pathologic conditions, the following data are offered: two subjects suffering from complete biliary obstruction due to carcinoma of the head of the pancreas showed no measurable quantity of urobilinogen excretion in the stool, and in two subjects suffering from familial hemolytic icterus in crisis, the values obtained were 1380 and 830 mg. per 100 grams of stool, respectively.*

SUMMARY

Using a 2.6 per cent solution of sodium hypochlorite as an oxidizing agent, a simple and accurate method is outlined for the determination of bile pigments in the urine and stool.

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* Complete data on serial observations of urobilinogen and bilirubin excretion in many pathologic conditions will be published later.

STERNAL MARROW MEGAKARYOCYTES IN HEALTH AND DISEASE*

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This report reviews the technics for enumerating megakaryocytes in bone marrow as reported in the literature, and introduces a modified procedure which is rapid, simple and accurate. Three technics for the enumeration of megakaryocytes in the bone marrow are in common use: smear, histologic section and counting chamber. The results of a number of investigators, as obtained with the various methods, are shown in Table 1.

METHODS AND MATERIALS

The Fuchs-Rosenthal modification of the Levy counting chamber having a volume of 3.2 cu. mm. was used in this study. One cc. of marrow was aspirated from the sternum by means of a 10 cc. syringe, then placed in a bottle containing 2 mg. of a previously dried mixture of ammonium and potassium oxalate (Heller and Paul anticoagulant).⁹ The bottle was rotated gently to prevent coagulation. Simultaneously, smears on three slides and four cover-slips were made from the marrow in the aspirating needle. With a white diluting pipet the oxalated marrow was drawn to the 1 mark and then diluted to the 10 mark with 5 per cent acetic acid containing methylene blue (1 cc. of 1 per cent methylene blue to 9 cc. of 5 per cent acetic acid). The acetic acid hemolyzes the non-nucleated erythrocytes, but leaves the leukocytes and immature erythroid elements clearly defined. The diluted marrow was then placed into the Fuchs-Rosenthal counting chamber. In the wet preparation all cells in the 256 squares were counted which had a diameter of 20 or more microns, an abundant granular cytoplasm and a high ratio of cytoplasm to nucleus. Two morphologic types of giant cells were found: multinucleated forms (polykaryocytes?) and those with a single lobulated nucleus. The cytoplasm was occasionally surrounded by a clear halo.

The following formula was used to calculate the number of megakaryocytes per cu. mm.:

$$\text{Megakaryocytes per cu. mm.} = \frac{\text{Megakaryocytes counted} \times \text{Dilution (10)}}{\text{Volume (3.2)}}$$

The total nucleated elements (T.N.E.) can be determined¹⁵ by filling an ordinary Levy-Neubauer counting chamber with 1:10 diluted marrow and counting the cells in five of the central squares, as for a routine red cell count.

$$\text{T. N. E. per cu. mm.} = \frac{\text{Number of cells counted} \times \text{Dilution (10)}}{\text{Volume (0.02)}}$$

* Received for publication, March 26, 1948.

When the number of nucleated cells is elevated, *i.e.*, above 200,000 per eu. mm., a 1:20 dilution of the marrow in the white cell pipet may be used in place of the 1:10 dilution.

Complete peripheral blood studies were made on normal persons as well as on patients with blood dyscrasias. Platelets were estimated by the methods of Rees and Ecker²⁰ and/or Reimann.²¹ The marrow findings were determined in

TABLE 1
NORMAL VALUES FOR MEGAKARYOCYTES OBTAINED BY VARIOUS AUTHORS

AUTHOR	PREPARATION	MEGAKARYOCYTES	AVERAGE NUMBER MEGAKARYOCYTES PER MILLION NUCLEATED CELLS
		<i>per cent</i>	
Dameshek and Miller ⁵	Smear	0.0099-0.0267	183
Limarzi <i>et al.</i> ¹²	Smear		58
Plum, C. M. ¹⁷	Smear	0.00-0.42	1900
Young and Osgood ³²	Smear	0.0-0.2	
de Renzi and Fuortes ⁶	Smear	0.0-2.7	5600
Segardahl ²³	Smear	0.0-0.55	300
Farber ⁷	Smear	0.26-1.2	
Kheifits ¹¹	Smear	0.0-1.0	
Gormsen ⁸	Smear		500
Henning and Keilhack ¹⁰	Smear	0.0-0.4	1000
Tempka and Braun ²⁷	Smear	2.16-4.0	
Arinkin ¹	Smear	0.6-6.1	
Mallarme ¹³	Smear	0.0-0.2	600
Plum, P. ¹⁸	Smear	0.0-0.4	1000
Vogel <i>et al.</i> ³⁰	Smear	0.0-0.8	2000
Vogel and Bassen ²³	Smear	0.0-0.6	1341
Stasney and Higgins ²⁶	"Abklatsch"		2200
Dameshek ⁴	Section	0.7-3.3	
Williams ³¹	Section	1.3-3.5	18,888
Custer ³	Section		2500
Nickerson and Sunderland ¹⁴	Section	0.246-423	3266
Barta ²	Section	0.07-0.83	
Sanchez Yllades ²²	Chamber		125
Smith ²⁵	Chamber	0.006-0.03	200
Shapiro and Bassen ²⁴	Chamber	0.0-0.21	435
Vogel and Bassen ²³	Chamber	0.0088-0.129	455
Pizzolato.....	Chamber	0.015-0.035	211

10 normal white persons studied at Charity Hospital. They included physicians, medical students and technicians whose ages ranged from 17 to 27. The result in each subject represented the average figure obtained from 2 to 4 chamber fillings. The number of megakaryocytes per eu. mm. for both sexes ranged from 6.3 to 22.0, and the average value was 13.1 (Table 2).

Megakaryocyte counts made on 300 patients with various blood dyscrasias have ranged from 0 to 187 per cu. mm. Aplastic anemia and the terminal stages of other blood dyscrasias yielded the lowest values and thrombocytopenic pur-

pura of Werlhof the highest value (Table 3). There was no definite correlation between the megakaryocyte counts, the total number of nucleated elements in the marrow and the peripheral blood platelet count.

To observe variation in sampling in another series of experiments, 1 cc. of marrow was aspirated from each of two sites in the sternum, about 2 to 3 cm. apart, from each of six patients (Table 4). Slight differences in values were noted. Although bleeding into the surrounding marrow is thought to occur, dilution of the myeloid elements was not obvious.¹⁶

The Fuchs-Rosenthal chamber method was compared with two frequently used procedures, the coverslip (0.87 sq. in. = 2.2 sq. cm.) method* and the slide

TABLE 2
NUMBER OF MEGAKARYOCYTES, TOTAL NUCLEATED ELEMENTS AND PLATELETS IN HEALTH

SEX	AGE	MEGAKARYOCYTES	NUCLEATED ELEMENTS IN THOUSANDS	PLATELETS IN THOUSANDS
		<i>per cu. mm.</i>	<i>per cu. mm.</i>	<i>per cu. mm.</i>
Males	23	15.6	66.5	200
	24	12.5	43.0	290
	27	22.0	87.0	350
	23	15.6	74.5	220
	22	15.6	43.1	300
	26	9.4	24.6	325
	Average.....	15.1	56.5	281
Females	22	6.3	35.0	200
	17	9.4	30.0	260
	19	12.5	40.0	300
	24	12.5	87.0	300
	Average.....	10.2	48.0	265
	Average (both sexes).....	13.1	53.1	275

(3 x 1 in. = 7.6 x 2.5 cm.) method. Five thousand nucleated cells were counted on each coverslip, and the number of megakaryocytes was expressed in relation to a million nucleated cells. Employing the slide method of Dameshek and Miller,⁵ the stained smear was covered with paper except for a rectangle 2.0 x 1.5 cm. (0.8 x 0.6 in.). This rectangle was placed just proximal to the feather edge. The megakaryocytes in this area were enumerated and expressed in proportion to a million nucleated cells. The total number of nucleated elements was computed at this site by counting the number of nucleated cells at three random horizontal areas embraced by the oil immersion lens, calculating the average count at the three areas and multiplying by 100 (Table 5). The coverslip method corresponded with only one result obtained by the counting chamber

* This method was recommended to the author by Dr. Joseph Stasney¹⁶ of Philadelphia.

TABLE 3

PATHOLOGIC CONDITIONS ACCOMPANIED BY ABNORMALLY LOW OR HIGH MEGAKARYOCYTE COUNTS

DIAGNOSIS	MEGAKARYOCYTES	NUCLEATED ELEMENTS IN THOUSANDS	PLATELETS IN THOUSANDS
	<i>per cu. mm.</i>	<i>per cu. mm.</i>	<i>per cu. mm.</i>
Lymphatic leukemia, chronic.....	0	30.5	70
Stem cell leukemia.....	0	55.0	40
Macrocytic anemia.....	0	30.0	110
Aplastic anemia.....	3	9.0	80
Aplastic anemia.....	0	27.0	90
Neutropenia, malignant.....	0	33.0	120
Plasma cell myeloma.....	0	24.5	140
Plasma cell myeloma.....	0	35.0	80
Thrombocytopenic purpura, secondary... ..	0	11.0	50
Septicemia, staphylococcic	3	25.5	100
Leukemoid reaction.....	3	200.0	87
Chronic myelogenous leukemia	0	230.0	130
Chronic myelogenous leukemia.....	3	502.0	150
Chronic lymphatic leukemia.....	0	245.5	90
Stem cell leukemia.....	0	216.0	110
Pernicious anemia.....	0	210.0	150
Macrocytic anemia, cause unknown.....	50.0	88.5	120
Macrocytic anemia, cause unknown	87.0	178.0	390
Macrocytic anemia, cause unknown.....	86.0	283.0	120
Sickle cell anemia.....	90.0	208.0	1050
Splenomegaly, idiopathic.....	65.5	132.5	70
Thrombocytopenic purpura, chronic idiopathic....	187.5	75.0	110
Hodgkin's disease.....	93.8	192.0	264
Septicemia, streptococcic.....	50.0	315.0	231
Septicemia, organism not isolated.....	50.0	215.5	100
Septicemia, staphylococcic.....	90.0	73.0	60

TABLE 4

PATHOLOGIC CONDITIONS FROM WHICH MARROW WAS ASPIRATED AT TWO SITES OF STERNUM

DIAGNOSIS	MEGAKARYOCYTES	NUCLEATED ELEMENTS IN THOUSANDS	PLATELETS IN THOUSANDS
	<i>per cu. mm.</i>	<i>per cu. mm.</i>	<i>per cu. mm.</i>
Sickle cell anemia	55.3	201.1	400
	37.5	214.0	
Hodgkin's disease	48.8	96.8	350
	73.7	308.0	
Bleeding peptic ulcer	28.1	72.0	375
	37.5	84.3	
Macrocytic anemia, cause unknown	9.4	25.1	200
	34.4	78.8	
Lymphosarcoma	7.5	12.5	225
	6.5	9.4	
Pernicious anemia	55.3	55.0	300
	43.8	40.8	

method, whereas the figures obtained with the slide method were in accord with four results of the chamber procedure. The results obtained with the coverslip and slide methods did not reveal any correlation.

DISCUSSION

An attempt was made to compare the Fuchs-Rosenthal chamber method of counting of megakaryocytes with other methods in common use. A common denominator was found in the ratio between the megakaryocytes and the total

TABLE 5

COMPARISON OF THE FUCHS-ROSENTHAL CHAMBER METHOD AND STAINED PREPARATIONS

DIAGNOSIS	NUMBER OF MEGAKARYOCYTES			
	Per Cu. Mm.	Per Million Nucleated Cells		
	Chamber Method	Chamber Method	Coverslip Method ^a	Slide Method
No disease.....	15.6 (43,000) ^b	363	400	0 (32,000) ^c
No disease.....	9.4 (24,600)	342	200	0 (17,300)
Fever, unknown cause.....	25.0 (48,800)	518	0	167 (191,900)
Macrocytic anemia, unknown cause	19.0 (72,000)	261	800	53 (131,230)
Macrocytic anemia, unknown cause	1.6 (29,800)	54	200	0 (23,200)
Chronic lymphatic leukemia..	4.7 (61,900)	76	600	29 (102,000)
Thrombocytopenic purpura (Werlhof).....	31.0 (138,000)	227	0	112 (151,200)
Generalized osteoporosis, unknown etiology.....	14.8 (43,700)	339	1400	347 (80,700)
Pernicious anemia (after liver treatment).	40.6 (101,500)	400	200	370 (73,000)
Lymphoepithelioma.....	26.6 (56,400)	472	2200	87 (95,600)

^a Calculated from a count of 5,000 nucleated cells.

^b The figures enclosed in parentheses represent the total nucleated elements per cu. mm., using the Levy counting chamber.

^c The figures enclosed in parentheses represent the estimated number of nucleated cells in an area 2.0 x 1.5 cm.

nucleated elements which was expressed as the number of megakaryocytes per million of nucleated cells. On this basis, the average number of megakaryocytes in ten normal persons studied was 13.1 per 53,100 nucleated cells. If the average of the total nucleated elements of the marrow of each of six additional normal volunteers,¹⁵ who had been studied before this technic was established, were added to this series of normal megakaryocyte counts, the computation would be expressed as 13.1 megakaryocytes per 61,980 nucleated cells or 211 megakaryocytes per million nucleated cells.

The results obtained corresponded closely with the values of Dameshek and Miller⁵ and with the values of some workers who use the chamber procedure.^{22, 25}

Some of the advantages and disadvantages of various technics may be mentioned. The chamber method gives a more uniform distribution of the

megakaryocytes, and the results are more uniform in multiple counts on a given sample. The count can be done in a few minutes, but should be made within one hour after aspiration. However, no significant changes were noted up to twenty-four hours when the marrow was kept at 5 C. Unfortunately, the finer cellular details are lost in the wet preparations. Other stains and variations of pH of the diluting fluid failed to produce greater details. Even the method of Randolph¹⁹ employing eosin, phloxine and methylene blue in propylene glycol as a hemolytic agent offered no advantage. The clear halo noted around some of the megakaryocytes with acetic acid diluting fluid was also found when propylene glycol was used as a hemolytic agent.

With the slide method, the megakaryocytes are concentrated in the feather end of the smear, and in the procedure of Dameshek and Miller⁵ the location of the rectangle determines the megakaryocyte count, the nearer to the feather edge the higher the count. Although many giant cells are frequently well preserved, their size renders them susceptible to trauma and fragmentation. The technic of examining the stained slide is extremely time-consuming, requiring not less than four hours. The method of Vaughan and Brockmyre²⁸ is less time-consuming but lacks uniformity, and at low power magnification young forms and small varieties are missed.

The coverslip preparations reveal irregularities in distribution as well as fragmentation from crushing. The time element is also excessive.

The histologic section requires many hours of preparation and a skillful tissue technician. Since many sections are studied, duplication in the enumeration of megakaryocytes may occur and early forms may not be discernible. The cellular details do not appear much superior to those in the chamber method.

SUMMARY

The literature indicates that there are many technics for counting megakaryocytes and that the results obtained by the different methods and by the same method in different hands vary greatly. The addition to the literature of still another procedure for megakaryocyte counts can only be justified by the simplicity and rapidity of technic and a reliability equal to or greater than the present methods. The use of a larger counting chamber with a smaller dilution of fluid reduces the personal and mechanical factors of error while taking advantage of the facility and speed of the counting chamber technic.

Acknowledgment. The author is indebted to Dr. Emma S. Moss and her technical staff for valuable assistance and to Dr. Herbert Derman and Dr. Ralph N. Baillif for valuable suggestions.

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9 ESTIMATION OF MEGAKARYOCYTE CONTENT OF ASPIRATED STERNAL MARROW*

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In spite of its importance, satisfactory methods for estimating the number of megakaryocytes in aspirated bone marrow have not been devised.^{2, 7, 29, 30}

Various methods in use are based on (1) inspection of stained smears of aspirated marrow, (2) hemocytometer counts on aspirated fluid marrow, and (3) examination of sections of marrow tissue. These methods are briefly reviewed.

The present paper is primarily concerned with a study aimed at determining whether or not there is correlation between estimates of megakaryocytes based on smears or hemocytometer counts, and those based on examination of serial sections of aspirated marrow particles. If the smear or hemocytometer methods were to give results which correlate with the more tedious and time-consuming section method, they could be used in preference.

REVIEW OF METHODS

Peabody²¹ stated that smears do not give a correct idea of the relative numbers of different cell types because of the tendency of the cells to adhere to each other. Kato¹⁴ did not attempt to determine the number of megakaryocytes in smears of aspirated sternal marrow in his series of 51 normal infants and children. According to Plum,²³ these large cells are "caught" by the tissue structures in the marrow, so that they are reduced below their true incidence in smears. The distribution of nucleated cells in direct smears of aspirated marrow is irregular, some cells being concentrated along the ends and lateral margins. The tendency for irregular distribution is particularly great in the case of megakaryocytes. Concentration technics,^{3, 17, 24} in which the nucleated cell layer in the centrifuge tube is removed and thoroughly mixed with plasma before smears are made, produce more uniform arrangement of nucleated cells, except that megakaryocytes remain more numerous at the thin edges and lateral margins of the smears. Valentine²⁹ does not consider the distribution of megakaryocytes in smears to be sufficiently uniform to allow a reasonably accurate estimate of their relative numbers. Nevertheless, certain authors have made use of stained smears for determining the megakaryocyte content of aspirated marrow.

Some have resorted to the simple technic of preparing a differential count of megakaryocytes in smears and express the number of megakaryocytes as a proportion of the nucleated cells.^{9-12, 16, 22, 23, 25-27, 32} As judged by reports on normal persons, this method is not very satisfactory. For example, Young and Osgood³⁵ observed that megakaryocytes were so few in marrow smears from

* Received for publication, July 15, 1948.

normal persons that they were omitted from the differential counts. Others have reported counts ranging from zero^{9, 12, 23, 26, 32} to as high as 6.1 per cent.¹ Segerdahl²⁵ reported a differential count of from 0.025 to 0.055 per cent. After trying several methods of megakaryocyte counting with poor results, Vaughan and Brockmyre³⁰ adopted the procedure of enumerating these elements in 50 low power fields at the edges of the smears. In our experience the frequency of megakaryocytes varies greatly among different smears from the same specimen of marrow, whether from the first drop of aspirated material or from the concentrated suspension of marrow cells.

A more elaborate device used by several authors^{8, 19} is the expression of the number of megakaryocytes as a proportion of the total number of nucleated cells in the smears, usually in terms of the number of megakaryocytes per million nucleated cells. Limarzi and Schleicher¹⁹ prepared smears of concentrated suspensions of marrow cells with an 18 mm. spreader, so that their smears were 18 mm. wide and approximately 30 mm. long. They counted all cells of the megakaryocytic series in the entire smear. The number found, multiplied by 0.6, gave the approximate number of megakaryocytes per 18 mm. square. The number of nucleated cells in this square was then determined. This was done by finding the average number of cells in ten oil immersion fields and multiplying this result by 25,600 which represented the number of oil immersion fields in an 18 mm. square area. The result was then corrected for megakaryocytes per million nucleated bone marrow cells. In ten normal individuals there were 52.2 megakaryocytes per 18 mm. square, or 58.8 megakaryocytes per million nucleated cells. Others^{18, 28} follow the practice of enumerating the megakaryocytes in the 18 mm. square which includes the feather edge of the smear (and the majority of the megakaryocytes), with approximately the same results. The figures reported by Limarzi and Schleicher are significantly lower than those obtained by Dameshek and Miller⁸ who used a fundamentally similar method. The latter reported a calculated incidence of 99.9 to 266.9 megakaryocytes per million nucleated cells in ten normal persons.

In general, when marrow contains large numbers of megakaryocytes, as revealed in sections of aspirated marrow particles, the smears also contain many of these cells. Occasionally, however, smears contain few of the cells in spite of a high incidence in the marrow sections.² Methods based on examination of smears are unsatisfactory for quantitative studies for the following reasons: (1) Aspirated marrow is unavoidably diluted with sinusoidal blood to varying degrees; (2) the distribution of megakaryocytes on smears is irregular; (3) the total nucleated cell content of bone marrow is variable from patient to patient; (4) the incidence of megakaryocytes may vary independently of the incidence of other nucleated cells. It must be concluded that quantification of megakaryocytes in samples of aspirated marrow on the basis of the total nucleated cell content of smears merely creates a false image of numerical accuracy.

Hemocytometer counts of megakaryocytes are subject to error introduced by variable dilution with sinusoidal blood. Shapiro and Bassen²⁶ observed a range of zero to 264 megakaryocytes per cu. mm. of aspirated marrow fluid from 35

normal infants. Even greater variation in 41 normal children was reported by Vogel and Bassen,³¹ the range being zero to 880 megakaryocytes per cu. mm. Yllades³⁴ stated that counts of 10 to 20 per cu. mm. are to be expected in normal persons. An advantage of hemocytometer counts is the ease with which they can be made. Furthermore, the cells are evenly distributed in the fluid suspension of the counting chamber. Finally, the results represent absolute values related to a unit quantity of marrow, rather than relative values related to the variable base of total nucleated cells. Unfortunately, as will be shown below, the method does not produce results which are always reliable.

The importance of studying sections of marrow for determining its structure, and especially for enumerating infrequently appearing cells, has been emphasized by others.^{7, 13, 15, 21} Casual inspection of a few sections is of far less value than a systematic enumeration of megakaryocytes with a standardized, easily duplicated procedure. Bunting⁶ expressed the incidence of megakaryocytes in terms of their numbers per square millimeter of sectioned marrow. Although such values are arbitrary, they are independent of other variables, such as fat and leukocyte content, vascularity or bone structure. Such values can be compared with counts made from control material studied by identical means. Custer⁷ used a ruled ocular (Zeiss Okularnetzmikrom) to divide the fields into segments. Later, Krumbhaar and Custer¹⁵ determined the incidence of megakaryocytes in 200 consecutive fields. Then all cells in each of 10 fields were counted; the number of megakaryocytes in 200 fields (*i.e.*, a total of approximately 12,000 cells) was recorded and the percentage calculated. Encroachment of fat cells, bone trabeculae and blood sinuses on the fields was avoided as much as possible. Using this method, Nickerson and Sunderland²⁰ reported an incidence of 0.246 to 0.370 per cent of 24,000 nucleated cells seen in 400 fields in 9 normal persons.

Unfortunately, such data indicate the relative frequency of megakaryocytes only in the cellular parts of the marrow samples. In various clinical conditions the total nucleated cell content and the incidence of megakaryocytes may vary independently. Relative values are less informative than absolute values which express numerically the incidence of megakaryocytes per unit area of bone marrow section. By studying cellular areas and ignoring relatively hypocellular parts, the estimate of megakaryocytes is artificially altered. Williams³³ counted megakaryocytes in 100 microscopic fields at a magnification of 400. Only the more cellular areas were examined. The author then stated, "Since the volume of a cylinder equals $\pi r^2 h$, if r is the radius of the microscopic field, measured by a stage micrometer, and h is the thickness of the section, which is approximately 5 microns, the volume of 100 such cylinders equals $0.165^2 \times 0.005 \pi \times 100$ or 0.043. Then the number of megakaryocytes per cubic millimeter of marrow equals $\frac{\text{number of megakaryocytes in 100 fields}}{0.043}$." Since these calculated results

are admittedly arbitrary and without significance unless used in comparison with controls, we see no particular advantage in the calculation, as the values observed by area are merely multiplied by the constant, $\frac{1}{0.043}$. Furthermore, the method

ignores the fact that hypocellular regions may contain a normal, reduced or increased number of megakaryocytes.

MATERIAL AND METHODS

This study is based on enumeration of megakaryocytes in smears, fluid suspension of unstained marrow cells, and sections of marrow particles obtained by sternal aspiration in 69 patients with various diseases. In each case the material was obtained and processed by methods described in detail elsewhere.³ In brief, these consisted of the preparation of smears from the first drop of aspirated marrow and from the suspension of marrow cells in heparinized plasma after centrifugation (concentrate-smears), and serial sections of marrow particles. In addition, some of the aspirated material was diluted in Türk's fluid (glacial acetic acid 3.0 ml., distilled water 300.0 ml., 1 per cent aqueous gentian violet 3.0 ml.) and subjected to counting in the hemocytometer. The smears were stained in Wright's fluid. The terminal 18 mm. square area (which includes the feather edge of the smear) of several slides from each case was scanned and the numbers of cells belonging to the megakaryocytic series was determined, averaged and recorded. The samples of heparinized fluid suspension of marrow cells, diluted from 1:5 to 1:20, depending on the cellularity of the marrow, were scanned in the hemocytometer counting chamber and the number of megakaryocytes was expressed in terms of their incidence per eubic millimeter of aspirated fluid. Marrow particles, fixed in formalin and stained with hematoxylin and eosin, were sectioned serially at 6 microns thickness. Every tenth section was examined. The optical system consisted of a 44X objective and 15X ocular equipped with a Whipple eyepiece disc which overlay a conveniently-sized square field. Whole consecutive fields were examined, regardless of their structure or cellularity, so long as they consisted of actual bone marrow. Preliminary studies had indicated that examination of 50 fields yielded reasonably constant results in a given case.^{4, 5} Consequently, the total number of megakaryocytic cells encountered in 50 such fields was determined and recorded. The values obtained by the three different methods were then compared, for each case, and charted as points in the scatter diagrams below.

RESULTS

Figure 1 indicates the relationship between megakaryocyte counts on concentrate smears and hemocytometer counts on the corresponding samples of fluid aspirated marrow in 41 cases. It is noteworthy that, although there appears to be some grouping of points at low values, when either the chamber counts or smear counts are relatively high, the points are scattered without correlation. In other words, when the 18 mm. area of the smear contains relatively few megakaryocytes (6 to 37), the chamber counts are usually low (0.2 to 5.5 per cu. mm.) although occasional counts from 10.6 to 34.6 per cu. mm. are also observed. With smear counts over 50 per 18 mm. area the chamber counts vary between 1.4 and 100 per cu. mm. The smears, therefore, do not provide a very useful medium for estimating the megakaryocyte content of aspirated fluid. Similar

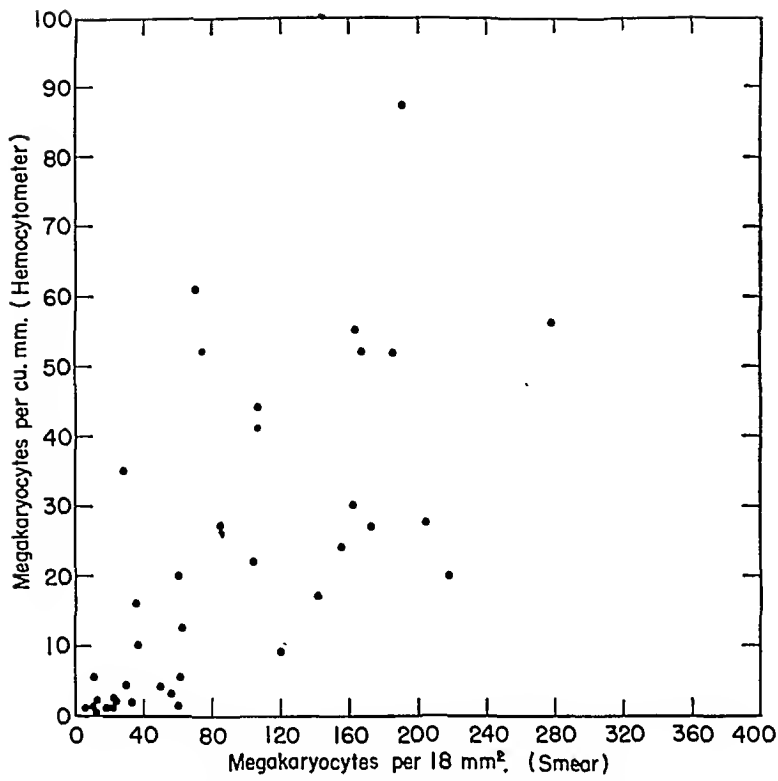


FIG. 1. The scatter diagram illustrates the relationship between megakaryocyte estimations based on smears and hemocytometer counts of aspirated sternal marrow in 41 cases.

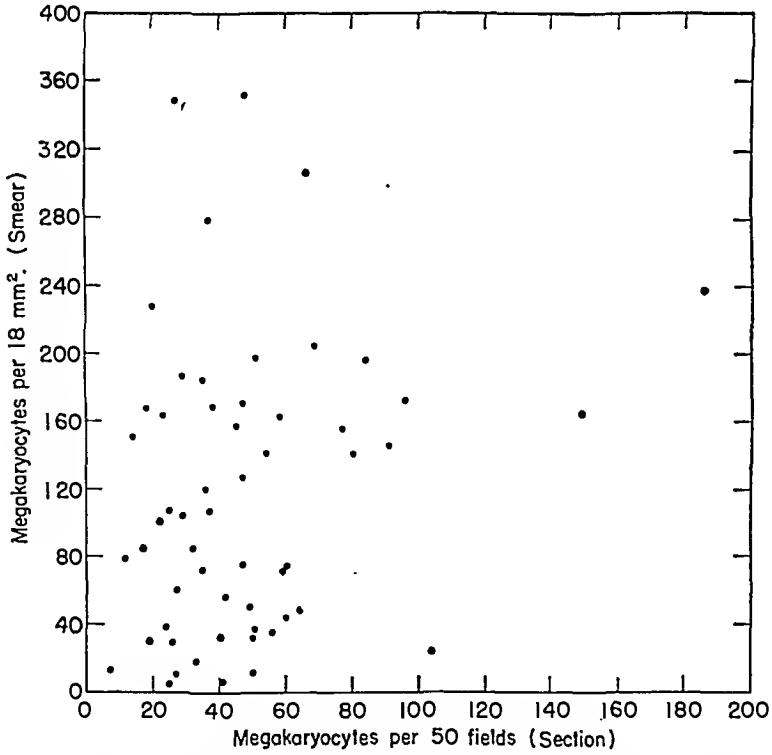


FIG. 2. The scatter diagram illustrates the relationship between megakaryocyte estimations based on smears and counts made from sections of aspirated sternal marrow in 58 cases.

results were obtained when smears from the first drop of aspirated marrow were used.

Figure 2 indicates the relationship between the megakaryocyte counts on concentrate smears and those based on examination of serial sections of marrow particles. Fifty-eight cases were available for this comparison. In the chart the points are scattered without correlation. Smears which contained less than 40 megakaryocytes per 18 mm. square were obtained from patients whose marrow sections yielded counts varying between 7 and 100 megakaryocytes per 50 fields. Likewise, smears with counts over 40 were obtained from patients whose sections yielded values between 12 and 186 per 50 fields. Smears, therefore, do

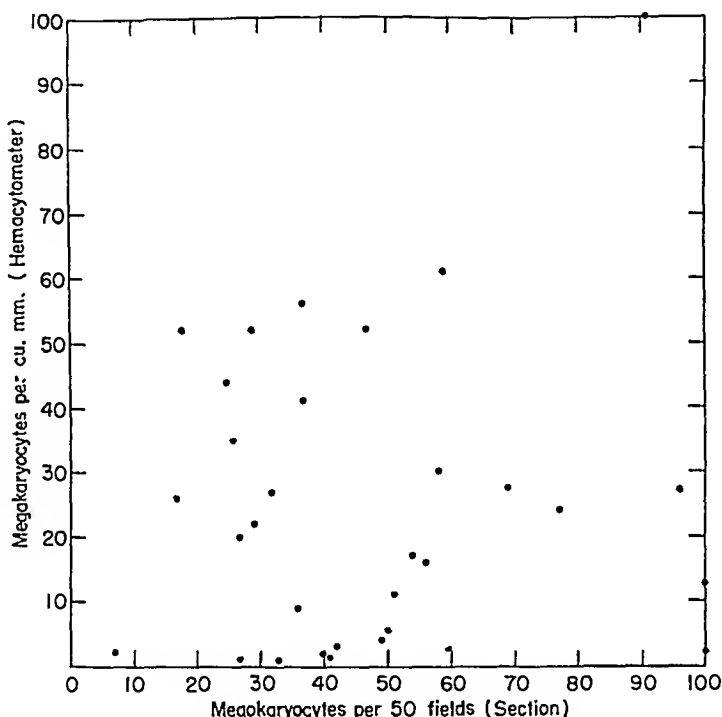


FIG. 3. The scatter diagram illustrates the relationship between megakaryocyte estimations based on sections and hemocytometer counts of aspirated sternal marrow in 32 cases.

not serve as satisfactory specimens for predicting the incidence of megakaryocytes in sections of aspirated marrow particles.

Figure 3 indicates the relationship between counts based on sections and those obtained by the hemocytometer method in 32 cases. The points are scattered diffusely. There is no evidence that chamber counts are satisfactory for predicting the incidence of megakaryocytes in sections of marrow.

DISCUSSION

Neither the smear nor chamber count method yields results which correlate with those obtained by study of actual marrow tissue sections and, therefore, they must be discarded for quantitative investigation of megakaryocytes in samples of aspirated sternal bone marrow. The explanation for the variable results

obtained by the smear or chamber methods has been discussed above. It is true that smears containing large numbers of megakaryocytes are likely to come from marrows having large numbers of these cells. Similarly, high chamber counts usually mean that the marrow contains many megakaryocytes. However, the lack of correlation between such counts and those obtained from sections of marrow tissue means that, for any given case, smear or chamber counts may be unreliable. Instances of low counts obtained by the smear or chamber methods in cases with high megakaryocyte content, as revealed in marrow sections, are disclosed in the scatter diagrams. Since the error of underestimating the megakaryocyte content of aspirated samples may be of clinical importance, especially when the question of splenectomy for thrombocytopenic purpura is presented, examination of marrow sections should not be omitted whenever the chamber or smear methods yield values suggestive of decreased megakaryocytopoiesis.

It must be emphasized that even the count in a section produces arbitrary values which cannot be converted into terms expressive of the actual number of megakaryocytes per unit volume of marrow. Hence, all such counts obtained from patients must be compared with counts made by identical means from suitable control cases.

SUMMARY

1. A review of present methods for estimating the megakaryocyte content of aspirated marrow reveals that none is satisfactory.

2. Various procedures in use are based on examination of stained smears, hemocytometer counts on aspirated fluid marrow, and examination of sections of marrow tissue.

3. The present study demonstrates no correlation between results obtained from study of smears and those obtained by the hemocytometer method. Furthermore, there is no evidence of correlation between counts from smears and sections, and finally, there is no evidence of correlation between counts based on examination of sections or the hemocytometer method.

4. For quantitative study of megakaryocytes in aspirated marrow, the use of sections of aspirated marrow particles is recommended. The values obtained must be compared with suitable controls studied by identical methods.

Acknowledgments. Technical assistance was furnished by the Anemia Laboratory, Out-patient Department, Harper Hospital, Detroit, Michigan. Thanks are due Miss Patricia Carlisle for assistance in preparation of materials for this study.

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THE DETECTION OF BARBITURIC ACID DERIVATIVES IN URINE

A RAPID QUALITATIVE TEST*

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The barbituric acid derivatives have become preferred agents in suicidal attempts. Each hospital has its share of cases of coma, and an increasing number of these is due to barbiturate intoxication. In such cases it is important to make the diagnosis early so that proper analeptic therapy may be instituted without delay. It is the purpose of this paper to describe a simple, rapid diagnostic laboratory test for the detection of barbiturate in the urine.

Romanova⁷ described a reaction produced by veronal and copper salts in which a blue precipitate formed. Zwikker⁹ separated barbital derivatives from crude extracts by precipitation with copper salts and pyridine and identified the barbiturate by determination of the melting point following treatment with dilute sulfuric acid. This author also described a specific test for barbital and its homologues which involved the formation of a deep blue cobalt-barium complex. It was pointed out¹⁰ that barbital forms coordination compounds with cobalt as the central atom and ammonium or an organic base held by residual valencies. Thus, a red dibarbitalcobaltodiamine has been isolated and identified.

Oettel⁵ described a method for the approximate quantitative estimation of the barbital content of urine and drugs based on the blue color obtained with cobalt acetate and lithium hydroxide in absolute methanol. Koppányi *et al.*⁴ prefer cobalt acetate and isopropylamine in their colorimetric chemical assay; they isolated the barbiturate from urine by precipitation of impurities by means of copper salts and alkali. Koppányi's cobalt acetate-isopropylamine test was adapted to the spectrophotometric estimation of barbital by Green *et al.*² who also rendered the determination more precise by the use of Lloyd's reagent which adsorbs urinary pigments and other chromogens without removing the barbiturates.

Raventos⁶ employed a method of continuous extraction with peroxide-free ether, followed by a purification of the extract and a separation of barbituric from thiobarbituric acid by chromatographing on activated alumina columns. The barbiturate was determined by Koppányi's colorimetric method.

Our method is a modification of the cobalt color reaction which differs from methods previously described in the following respects: (1) Ether is used as the solvent instead of chloroform; (2) a wetting agent is employed to break up any emulsion formed between urine and the solvent; (3) activated charcoal is used as a decolorizer; (4) the method emphasizes speed and simplicity rather than strict quantitative precision.

* This investigation was supported in part by special gifts from Mrs. S. Prentiss Baldwin. Received for publication, June 5, 1948.

METHOD

Acidify 100 ml. of urine to pH 4.0 (Nitrazine paper) with dilute sulfuric acid (one volume concentrated acid to six volumes of water). In a separatory funnel extract twice with 75 ml. portions of absolute ether,* shaking for 30 seconds during each extraction. If an emulsion forms which will not separate on standing, add a suitable wetting agent,† rotate the funnel gently and wait for the layers to separate. Combine the ether extracts in a 250 ml. Erlenmeyer flask. Add activated charcoal (Nuchar C-250, 2 Gm.) and anhydrous sodium sulfate (5 Gm.) and shake vigorously for one minute. Allow to stand for three minutes, then decant the clear ether solution through filter paper, collecting the filtrate in a separatory funnel. Wash the activated charcoal-sodium sulfate residue with 25 ml. of absolute ether and transfer the entire mixture to the filter paper. Evaporate the filtrate to 25–50 ml. on the steam bath, allowing the solution to drain from the separatory funnel into a 100 ml. beaker. Finally, evaporate to 2 ml. in a suitably calibrated tube.

To the final 2 ml. of concentrated ether extract add 0.5 per cent lithium hydroxide in absolute methanol (5 drops or 0.2 ml.). If more than 1 mg. of barbituric acid is present a white precipitate will form. Add, drop by drop, 0.2 per cent cobaltous acetate in absolute methanol until any blue-violet color reaches its maximum intensity (do not exceed 10 drops or 0.4 ml.). A distinct blue-violet color as compared with a control test carried out on 2 ml. of absolute ether indicates the presence of a barbituric acid derivative in the original urine sample. A rich green color indicates the presence of a thiobarbiturate such as Pentothal sodium.

RESULTS

The following barbituric acid derivatives were tested by this method: barbital, Amytal, probarbital (Ipral), Neonal, Ortol, thiopental (Pentothal sodium), pentobarbital (Nembutal), phenobarbital, Phanodorn, hexobarbital (Evipal), alurate, Dial, Seconal, Sandoptal, Nostal, Pernoston, and vinbarbital (Delvinal). All gave the characteristic blue-violet color, except Pentothal sodium, which produced a rich green color.

The use of ether as the extracting solvent is recommended because of its high volatility. Thus, considerable time is saved in evaporation of the solvent.

The extraction of urinary constituents with organic solvents, such as ether, often results in the formation of an emulsion which sometimes will not separate for many hours. The layers may be separated by centrifugation or by the addition of large amounts of ammonium sulfate, but of much greater convenience and simplicity is the addition of a wetting agent. This technic results in rapid and complete separation of the two layers.

The original ether extract is discolored with urinary pigments which interfere

* The ether must be free from peroxides which interfere with development of the final blue color.

† We find that 1 gram or more of "Dreft" (Proctor and Gamble Co., Cincinnati, Ohio) is excellent for this purpose.

with the detection of the blue color produced in the cobalt reaction. Attempts were made to remove the pigment by adsorbing substances such as Lloyd's reagent and activated alumina. These substances failed to remove the color completely and consistently; however, activated charcoal (Nuchar C-250) decolorized the ether extract efficiently and is recommended for this purpose. It is possible that a certain amount of barbiturate may be adsorbed by the charcoal.³ This would reduce the sensitivity of the procedure to some extent but, it is felt, would not seriously limit the usefulness of the method.

The characteristic blue-violet color is produced with as little as 0.2 mg. of barbital, phenobarbital, or pentobarbital (in 2 ml. of absolute ether). This is considered the practical limit of sensitivity of the cobalt color test as carried out by our method.

Two (2.0) mg. of barbital, phenobarbital or pentobarbital added to urine was recovered in sufficient amounts consistently to give a strong positive reaction. Of these barbiturates, 0.5 mg. at times gave a positive test and at times only a questionably positive result.

The analysis of urine for barbiturate by the method described can be carried out in about one hour.

DISCUSSION

In all patients admitted to a hospital in coma it is important to consider the possibility of acute barbiturate intoxication. Without a history of ingestion of barbiturate the diagnosis is difficult, the physical examination may be of little value, except to indicate a severe central nervous system depression. Under these conditions a positive diagnosis cannot be made without the recovery and identification of barbiturate in the urine, blood or stomach contents.

In this connection the quickest possible answer to the question whether any barbituric acid derivative at all is present is more important than a determination of the absolute amount and the kind of substituted barbituric acid. It is true that certain barbiturates appear in the urine even when taken in therapeutic quantities, but this is limited to barbital (60 to 80 per cent elimination by the kidneys) and phenobarbital (20 to 30 per cent elimination) whereas the other, shorter acting barbiturates are nearly completely destroyed by the liver.^{1,8} Therefore, these barbiturates may not appear in the urines, even if toxic amounts have been ingested or injected.

With a strongly positive qualitative test for barbiturate in the urine, there is strong presumptive evidence for the conclusion that the coma is due to barbiturate intoxication.

SUMMARY

A rapid, convenient test for the detection of barbituric acid derivatives in the urine is described.

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A SIMPLIFIED TECHNIC FOR PRESERVATION OF ANATOMIC SPECIMENS IN PLASTIC*

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The use of modern plastics in the preservation of biologic specimens was first recorded in 1937 by Hibben² and Sando.⁵ Further development of this medium has been concerned with the preservation of small osseous or chitinous specimens. The large, moist anatomic specimens present a greater problem.

Darrah¹ described a very simple method of wrapping and sealing formalin-fixed whole brains and brain slices in "Pliofilm" in such a way that they could be left exposed for many months at a time. This method would probably be equally satisfactory for other gross specimens. Rosett,⁴ as well as Mettler and Mettler,³ developed the Bakelite technic of embedding. Specimens so preserved are hard and of lasting quality, but inasmuch as the natural color and structural details are lost, they have not been satisfactory. More recently Strumia and Hershey⁶ have applied the ethyl methacrylate method with some modification to the larger moist anatomic specimens with good results but, again, the technic is elaborate, time-consuming and only warranted for valuable permanent museum pieces.

A plastic method, used by us at The Brooklyn Hospital for the preparation of anatomic and pathologic specimens, has proved to be much more simple and of great value for conference and classroom demonstrations. Specimens prepared by us one year ago have remained in good condition.

Specimens obtained directly from the operating room or from the autopsy table are fixed by the Kaiserling method to preserve the natural color. Simple formalin-fixed specimens are equally suitable, but do not make as colorful an exhibit. After adequate fixation by either of the above methods the specimen is dried gently with paper towels and suspended for dipping into the plastic. We have found one or two four-ply strands of ordinary cotton thread, knotted at their ends, sufficient to bear the weight of the ordinary specimen together with its coats of plastic. An extra long needle is of value to thread the strands through the specimens. In some cases we have inserted wooden applicators at inconspicuous places to give the specimen added strength.

We have used the crystalline powder of butyl methacrylate polymer P4. (This plastic is obtainable from the DuPont Company, Wilmington, Delaware.) The methacrylate is dissolved in xylol in the proportions of 200 Gm. of plastic to 400 cc. of xylol. This makes a solution of thick syrupy consistency. If, upon standing, the solution becomes too thick unsightly bubbles of evaporating xylol may be trapped in the coating. The specimen is dipped in the plastic, and before removal, all clinging bubbles are brushed off with a small paint brush. The plastic coat dries in the air within fifteen to twenty minutes, and the dipping

* Received for publication, April 8, 1948.



FIG. 1. Section of normal liver. The small white spots are blood vessels in cross section.



FIG. 2. Slices of brain and hemisection of normal brain.

is repeated. Ten to fifteen dippings will form a coat 5 to 8 mm. thick which has proved sufficient for our purposes. The specimen is then hung to dry in the air for at least one week to prevent tackiness or indentation marks. The plastic-coated threads are then cut off about one fourth of an inch from the specimen, and it is ready for dry storage, museum exhibition, or conference and classroom handling.

The specimen of half of a brain (Fig. 2) required somewhat more time and care, but is well preserved one year after preparation, and appears durable.

SUMMARY

Dipping of fixed anatomic specimens in a butyl methacrylate-xylol mixture has proved to be a simple method of preservation. Specimens so prepared have not become dehydrated or discolored in the year that this method has been in use. The convenience and cleanliness in handling odorless, dry preparations makes the method ideal for use in teaching and conferences.

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A MODIFIED AND IMPROVED STERNAL PUNCTURE NEEDLE*

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The present simple method of obtaining bone marrow from the sternum during life for study and diagnostic purposes was first introduced by Arinkin in 1929. With its increasing popularity, the technics and types of sternal puncture needles¹ have been modified in various ways to suit the individual needs of the investigator. Of the many types of needles, the one devised by Klima and Rosegger^{2, 3} in Germany in 1935 has had more general acceptance. Two investigators⁶ in this country have been able to use this needle in its original form with a syringe of domestic manufacture by adding a suitable adapter. The original needle consisted of a short (approximately 3½ inches in length) 16-gauge lumbar puncture needle, an obturating stylet and an adjustable guard for the needle shaft. The stylet was set into the hub of the needle and prevented from rotating by a small pin-insert. The adjustable guard which was machine-screwed to the base of the needle was of little aid in controlling the depth of the puncture while going through the anterior plate of the sternum. The guard actually serves as a support for the index finger and thumb, enables the operator to obtain a firm grip of the needle and facilitates the downward pressure necessary to enter the marrow cavity.

Although several difficulties were encountered in the use of the original Klima and Rosegger Needle, war-time restrictions prevented any further modification or improvement in the needle. One of the unsatisfactory features of the needle concerns the stylet, which through wear becomes loose and thus may be unseated while entering the bony table of the sternum. To reseat the stylet, it was found necessary, at times, to apply considerable pressure. Often in puncturing a very sclerotic bone, the stylet may become elevated allowing spicules of bone marrow or clotted blood to enter the lumen, thus blocking it. This often necessitates removal of the needle and a second attempt at sternal puncture, with resultant discomfort to the patient and operator.

The needle† has been modified and improved by the following additional features: (1) The stylet has been made larger and a knurled edge incorporated to insure an easy grip; (2) the stylet, by means of a special locking device, is held rigidly in place enabling the needle to enter any type of bone without fear of displacing the obturator. The tightly fitting stylet prevents procaine or peripheral blood from getting into the lumen and prevents spicules of bone marrow or clotted blood from blocking the lumen (Fig. 1); (3) as a part of the locking device, a Luer-Lok hub is set into the needle which allows either the conventional

* Received for publication, July 14, 1948.

† This needle, called the University of Illinois Sternal Needle, is manufactured by V. Mueller and Company, 408 South Honore Street, Chicago 12, Illinois.

Luer glass tip or Luer-Lok tipped syringe device to be used. It is preferred that the Luer-Lok tipped syringe be used to avoid breaking the glass tip on the needle hub. Thus, aspiration or infusion technic may be used if desired. Figure 2

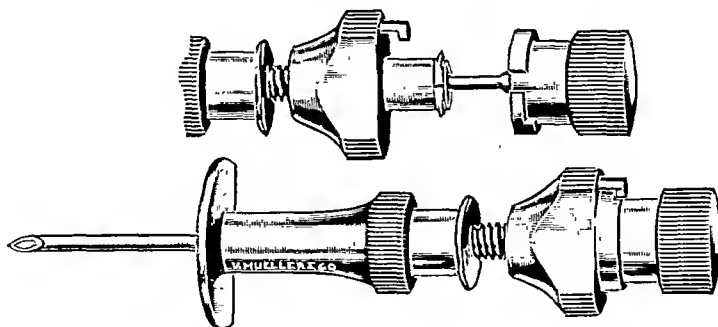


FIG. 1. Two views of the sternal puncture showing the special locking device of the needle and stylet and the Luer-Lok hub set into the needle. (Courtesy of V. Mueller and Company, Chicago)

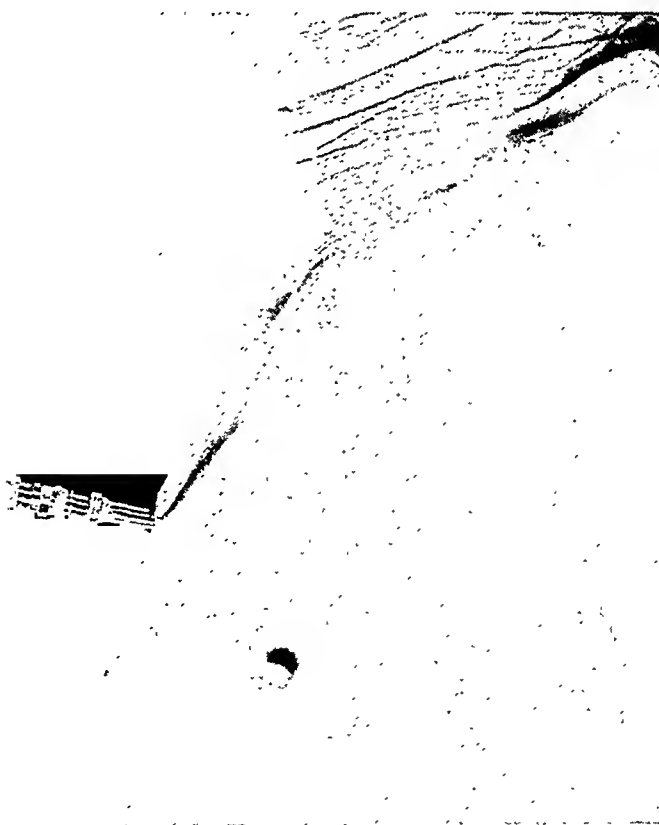


FIG. 2. The modified and improved sternal needle in position in the sternum at the second costal inter-space. Note that the sternal needle *in situ* should be perpendicular to the sternum.

shows the sternal needle in the sternum of a patient with the stylet locked into the closed position.

The method of sternal puncture and preparation of marrow specimen with

description of the apparatus and procedure has been previously reported by one of the authors.^{4, 5} Because of the design and size of the needle it can be easily and readily used in punctures of the vertebral spine, femur or tibia.

SUMMARY

A modified and improved sternal puncture needle is described which incorporates a number of new features, one of which is a lock which holds the stylet in place and prevents its displacement or blocking of the needle by a particle of bone or clotted blood.

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A RAPID METHOD OF FILLING AND CLEANING WINTROBE HEMATOCRIT TUBES*

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The methods which have been employed for filling the Wintrobe hematocrit tube are not only time-consuming but tedious. When glass pipets are used there is considerable breakage. The following method, which has been employed for over two years, minimizes the danger of breakage. It is also advantageous in eliminating the need for making new pipets. In addition, this method is much more rapid than other methods in that the tubes can be filled or cleaned in a matter of seconds.

The needle is detached from a blood-filled syringe and replaced with a 5-inch 13-gauge needle. The latter is then inserted into the Wintrobe tube which is held vertically and filled with blood to the proper mark. Any excess may be removed by changing to a smaller bore needle (21 or 22 gauge) and withdrawing the desired amount. The tubes are then centrifuged and the reading is made in the usual manner.

The tubes are cleaned by employing a water-filled 20 cc. syringe fitted with a 5-inch 13-gauge needle. The needle is inserted to the bottom of the tube and by forcing water through the syringe, the material contained within the tube is expelled. Any water remaining in the tube is removed by aspiration. The tubes are then dried in the usual manner.

* Received for publication, July 19, 1948.

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OBSERVATIONS ON THE RARE GENES R^z AND r^{v*}

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In 1943, with the aid of the three Rh antisera, anti-Rh₀, anti-rh' and anti-rh'', the existence of eight Rh blood types was demonstrated.¹⁰ To account for the hereditary transmission of the eight types a minimum of six allelic genes had to be postulated, namely, R^0 , R^1 , R^2 , r' , r'' and r . Family and statistical data have been published which support this theory.^{1, 3, 6, 11, 12, 13, 14}

The existence of "double action" genes, R^1 (or $R^{0'}$) and R^2 (or $R^{0''}$), suggested the possible existence also of a "double action" gene, r' , and even of a "triple action" gene, $R^{0'}$, but our early family studies failed to reveal their presence. About this time, Race and Taylor⁵ had encountered some rare individuals of type Rh₁Rh₂ who, contrary to the usual expectation, were hr'-negative, and family studies subsequently proved⁴ these individuals to be bearers of the gene $R^{0'}$, which was designated R^z for short. In studies¹⁵ on the distribution of the Rh-Hr types among American Indians, we found a relatively high incidence of 3 per cent of the gene R^z , which is so rare among Caucasians. Subsequently, Simmons *et al.*^{7, 8} found a similar incidence (3 to 6 per cent) among the racially related Papuans and Australian aborigines.

The other theoretically possible gene, r' , designated r^v by Race and Taylor, has been identified by Stancu, Clark and Snyder.⁹ We have recently encountered three families which illustrate the hereditary transmission of the genes R^z and r^v . In view of the extreme rarity of such cases, they seem sufficiently important to be reported in detail.

Case 1. This family was referred for antenatal Rh-Hr studies because the expectant mother was in her third pregnancy and had been found to be Rh-negative while her husband was Rh-positive. The first two children, a girl and a boy, respectively, were alive and well and neither had had neonatal jaundice or anemia. Grouping and Rh-Hr typing of the family gave the results shown in Table 1.

It will be seen that the father belongs to type Rh₁Rh₂; the most common genotype corresponding to this phenotype is R^1R^2 . In previous studies¹⁶ on 207 families with 1249 children, we encountered 83 families with parents rh \times Rh₁Rh₂. Among the 130 children in these families there were 69 of type Rh₁, 60 of type Rh₂, and only one of type rh, and the latter proved to be an illegitimate child. These results were to be expected if, in these 83 families, the type Rh₁Rh₂ parents all belonged to genotype R^1R^2 .

The family presented in Table 1 is unusual since the first child belongs to type rh and the second to type Rh₁Rh₂. To explain such findings, it is necessary to postulate that the father is carrying the rare gene R^z and belongs to genotype R^zr . Under this assumption, the father is heterozygous for the Rh₀ factor and

* Received for publication, September 3, 1948.

is, therefore, different from other type Rh₁Rh₂ persons who are ordinarily homozygous for the Rh₀ factor. One would expect that half the children of this couple would be Rh₀-negative (type rh) as the daughter actually was, and half would be Rh₀-positive (Rh₁Rh₂) as the son actually was, and the chances were, therefore, even that the new baby would be either type rh or type Rh₁Rh₂. When the baby was born, it was found to belong to group B, type M, type Rh₁-Rh₂.

The antenatal examination of the mother's blood included tests for Rh antibodies. These were negative and the anti-A titer was very low. Therefore, the prediction was made that the expected baby would not be erythroblastotic, whether it proved to be Rh-positive or Rh-negative. This prediction has also been confirmed, since the baby was entirely normal.

TABLE 1
RESULTS OF BLOOD GROUP AND RH-Hr TESTS IN CASE 1

BLOOD OF	GROUP AND SUBGROUP	M-N TYPE	Rh-Hr TYPE
Father.....	A ₁	M	Rh ₁ Rh ₂
Mother.....	B	MN	rh
Daughter.....	A ₁	M	rh
Son.....	A ₁ B	MN	Rh ₁ Rh ₂

TABLE 2
RESULTS OF BLOOD GROUPS AND RH-Hr TESTS IN CASE 2

BLOOD OF	GROUP AND SUBGROUP	M-N TYPE	Rh-Hr TYPE
Father.....	O	MN	rh'rh"
Mother.....	A ₂	M	rh
First child.....	O	M	rh

Case 2. This family was referred for antenatal Rh-Hr studies because of the following history. In 1942, the mother had a corrosive poisoning with hemorrhage from the esophagus, for which she was given several blood transfusions. Her first child, born in 1946, was alive and well, and showed no evidence of erythroblastosis. The mother was now pregnant for the second time. The family's blood groups and types are given in Table 2.

Persons of type rh'rh" may be assumed to belong to the genotype r'r" and, therefore, could not have a type rh child. Since the first child in this case does belong to type rh, the alternative is that either the first child is illegitimate, or else that the father is the bearer of the extremely rare gene r" and belongs to genotype r"r. The father had two brothers whose bloods were tested in order to throw further light on the problem. Both brothers belonged to group A₁, type MN and type rh'rh". The observation of the type rh'rh" in all three brothers is explained more plausibly by assuming that each possesses gene r" than by postulating that they all belong to genotype r'r", since the former requires them to have only one gene in common while the latter requires them to

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have two genes in common. The final proof that the father belonged to genotype $r^y r$ was provided when the second baby was born and proved to belong to group A₂, type MN, type rh'rh".

It may be of interest to discuss also the clinical aspects of this case. In view of the history that the mother had received several blood transfusions, detailed tests were made upon her serum for Rh antibodies, with the results shown in Table 3. These results show the presence of Rh sensitization of mild degree with antibodies of the univalent variety. There is evidence of a double sensitization, with an anti-Rh₀ glutinin (univalent antibody) with an average titer of 4 units

TABLE 3
TITRATION OF ANTIBODIES IN SERUM OF MOTHER IN CASE 2

TEST CELLS	TITER (IN UNITS) BY THE METHOD OF	
	Agglutination (Saline media)	Conglutination (Plasma-albumin)
Rh ₀	0	4
Rh ₁ Rh ₂	0	3
Rh ₂ Rh ₂	0	4
Rh ₁ rh.....	0	5
rh'rh.....	0	1½
rh"rh.....	0	0
rh'rh" (father).....	0	1½
rh.....	0	0

TABLE 4
RESULTS OF BLOOD GROUPING AND RH-Hr TESTS IN CASE 3

BLOOD OF	GROUP	M-N TYPE	Rh-Hr TYPE	
			Phenotype	Genotype
Father.....	O	MN	Rh ₁ Rh ₂	$R^z R^1$ or $R^z r'$
Mother.....	O	N	rh	rr
Second child.....	O	MN	Rh ₁ Rh ₂	$R^z r$

and an anti-rh' glutinin with an average titer of about 2 units. Of these two antibodies, only the anti-rh' glutinin could possibly hurt the baby, and even if the baby proved to be type rh'rh" it was felt that the manifestations would probably be mild or subclinical, in view of the low titer of the antibody. In fact, the baby showed only a mild jaundice on the fifth day, at which time its hemoglobin concentration was 17 Gm. per 100 ml. of blood. On the seventh day, when the jaundice had cleared and the hemoglobin was 17.5 Gm., the baby was sent home perfectly well.

Case 3. This family was referred for antenatal tests, with the following obstetrical history. The first pregnancy resulted in the birth of a male infant, delivered by breech delivery. The infant died after eight hours, from intracranial hemorrhage. The second pregnancy also yielded a male infant, who showed no jaundice or anemia and is alive and

well. The patient was now in her third pregnancy and the expected date of delivery was December 19, 1948. Grouping and Rh-Hr tests were carried out with the results given in Table 4.

This, evidently, is again a case illustrating the genetic transmission of the R^z gene. In this case, there is not enough information to determine the father's genotype exactly. While initially the maternal serum contained no Rh antibodies, subsequently Rh₀ glutinins appeared and, therefore, an erythroblastotic baby is to be expected.

COMMENT

While our routine tests at present include the use of anti-hr' and anti-hr'' serums as well as the three Rh antiserums, anti-Rh₀, anti-rh' and anti-rh'', it is apparent that the genes r'' and R^z in the two families described here were actually identified by the tests with the Rh antiserums alone, together with the dis-

TABLE 5
REACTIONS DETERMINED BY THE RH GENES, INCLUDING r'' AND R^z

GENE	REACTIONS WITH ANTI-Rh SERUMS			REACTIONS WITH ANTI-Hr SERUMS		
	Anti-rh'	Anti-rh''	Anti-Rh ₀	Anti-hr'	Anti-hr''	Anti-Hr ₀
r	—	—	—	+	+	+
r'	+	—	—	—	+	+
r''	—	+	—	+	—	+
r''	+	+	—	—	—	+
R^0	—	—	+	+	+	—
R^1	+	—	+	—	+	—
R^2	—	+	+	+	—	—
R^z	+	+	+	—	—	—

tribution of the Rh types in the families. It is necessary to emphasize this since some protagonists of the so-called CDE terminology have made much capital of the fact that our early work was done without Hr antiserums. These workers also assert that the Rh-Hr designations are unsatisfactory because the Hr factors are not included in the gene symbols. The fallacy of this argument is easily seen by consulting Table 5 in which the reactions determined by each of the genes is listed. It will be seen that each gene is completely identified by its reactions with the three Rh antiserums, because the reactions given by the Hr antiserums are reciprocal to those of the corresponding Rh antiserums. The situation is entirely analogous to the reciprocal relationship between the agglutinogens and agglutinins in the case of the four Landsteiner blood groups, yet none but the neophyte feels the need for designations such as group O, anti-A, anti-B; group A, anti-B, etc., when naming the blood groups. In fact, the CDE designations suffer the disadvantage of being redundant, so that, for example, instead of the simple designation "type rh", these workers use the name "small c, small d, small e, over small c, small d, small e."

Now that the existence of the rare genes r'' and R^z has been demonstrated

conclusively, and the existence of the antibody anti-Hr₀ has been established by Haberman *et al.*,² it is permissible to summarize the essential facts concerning the Rh-Hr blood types as shown in Table 6. As can be seen, a total of 27 pheno-

TABLE 6
THE 27 RH-HR TYPES AND THEIR 36 GENOTYPES*

DESIGNATIONS OF THE 8 Rh TYPES	REACTIONS WITH Rh SERUMS			DESIGNATIONS OF THE 27 Rh-Hr TYPES	REACTIONS WITH Hr SERUMS			POSSIBLE GENOTYPES
	Anti-Rh ₀	Anti-rh'	Anti-rh''		Anti-Hr ₀	Anti-hr'	Anti-hr''	
rh	—	—	—	rh	+	+	+	rr
rh'	—	+	—	$r'r'$	+	—	+	$r'y'$
				$r'r$	+	+	+	$r'y$
rh''	—	—	+	$r''r''$	+	+	—	$r''y''$
				$r''r$	+	+	+	$r''y$
rh'rh''	—	+	+	$r_y r_y$	+	—	—	$r^y r^y$
				$r_y r'$	+	—	+	$r^y r'$
				$r_y r''$	+	+	—	$r^y r''$
				$r_y r$	+	+	+	$r'y''$ and $r^y r$
Rh ₀	+	—	—	$R_0 R_0$	—	+	+	$R^0 R^0$
				$R_0 r$	+	+	+	$R^0 r$
Rh ₁	+	+	—	$R_1 R_1$	—	—	+	$R^1 R^1$
				$R_1 R_0$	—	+	+	$R^1 R^0$
				$R_1 r'$	+	—	+	$R^1 r'$
				$R_1 r$	+	+	+	$R^1 r$ and $R^0 r'$
Rh ₂	+	—	+	$R_2 R_2$	—	+	—	$R^2 R^2$
				$R_2 R_0$	—	+	+	$R^2 R^0$
				$R_2 r''$	+	+	—	$R^2 r''$
				$R_2 r$	+	+	+	$R^2 r$ and $R^0 r''$
Rh ₁ Rh ₂	+	+	+	$R_z R_z$	—	—	—	$R^z R^z$
				$R_z R_1$	—	—	+	$R^z R^1$
				$R_z R_2$	—	+	—	$R^z R^2$
				$R_z R_0$	—	+	+	$R^1 R^2$ and $R^z R^0$
				$R_z r_y$	+	—	—	$R^z r^y$
				$R_z r'$	+	—	+	$R^z r'$ and $R^1 r^y$
				$R_z r''$	+	+	—	$R^z r''$ and $R^2 r^y$
				$R_z r$	+	+	+	$R^z r$, $R^1 r''$, $R^2 r'$, and $R^0 r^y$

* Does not include the reactions of the rare serum anti-rh''' (anti-C^w), or the rare "intermediate" Rh factors.

types can be assumed to exist, corresponding to 36 genotypes, though many of the phenotypes and genotypes are quite rare. For the sake of simplicity the so-called "intermediate" genes have not been included in Table 6. The pheno-

type names for the 27 Rh-Hr types, besides being simple, are self-explanatory, since the first symbol represents the reactions with the three Rh antisera, while the second symbol represents the reactions with the Hr antisera.

SUMMARY

Three families are presented which demonstrate the existence and hereditary transmission of the rare genes R^z and r^y . The established facts concerning the Rh-Hr type have been summarized in a table indicating the existence of 27 phenotypes with 36 corresponding genotypes.

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NON-ERYTHROBLASTOTIC HYDROPS FETALIS

RECURRING IN ASSOCIATION WITH TOXEMIA OF PREGNANCY*

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It is generally accepted that hydrops fetalis represents one of the clinical forms of hemolytic disease of the newborn. In almost all cases of hydrops fetalis recently reported in the literature,^{4, 5, 7} incompatibility in the Rh factor between mother and fetus as well as signs of maternal immunization have been observed. Even in those cases of hydrops fetalis where both mother and child are Rh-positive, maternal iso-immunization could be established following the recent developments in the study of Rh antigens and improvement in the detection of immune antibodies.⁸

However, before the discovery of antigenic incompatibility between mother and fetus as the cause of erythroblastosis fetalis, hydrops fetalis was related to various causes, such as fetal malformation and toxemia of pregnancy. In over one-third of all cases of hydrops fetalis, pre-eclamptic toxemia has been observed in the mothers.^{1, 3}

We wish to report on a patient who delivered two hydropic infants in her first and fourth deliveries, while the other two pregnancies terminated with the delivery of normal children. No evidence of antigenic incompatibility between mother and fetus, maternal iso-immunization or signs of fetal erythroblastosis were found. This observation is of interest since it points to the existence of two different etiologic groups of hydrops fetalis: one form probably accounting for the great majority of cases of hydrops fetalis, being caused by erythroblastosis, and the other, a non-erythroblastotic form, resulting from other causes, such as toxemia of pregnancy. Of course, no signs of Rh incompatibility or maternal iso-immunization are present in this second group.

REPORT OF CASE

S. M., aged 34, a Jewish woman of oriental origin, was admitted to the gynecologic department of the Hadassah University Hospital in January 1948, because of pre-eclamptic toxemia in the eighth month of her fourth pregnancy.

Her past obstetric history is as follows: In May 1940, in the ninth month of her first pregnancy, the patient was admitted to this hospital because of toxemia of pregnancy characterized by severe edema of the legs, abdomen and face, and albuminuria, and hypertension of 150/80. No fetal heart tones could be heard. The patient related that she did not feel fetal movements for at least one day prior to admission. Blood studies revealed urea 24 mg., sugar 98 mg., uric acid 7 mg., total proteins 4.68 Gm., albumin 2.58 Gm. and globulin 2.1 Gm. per 100 ml. The Wassermann and Kahn tests were negative. No Rh determinations were made then (in 1940). Urinalyses revealed albuminuria of 2 per cent. The ocular fundi showed arteriolar constriction. Three days after admission the patient

* Received for publication, August 23, 1948.

delivered a macerated hydropic male fetus weighing 2980 Gm. and a hydropic placenta weighing 980 Gm. The postmortem examination of the fetus showed generalized edema, normal-sized liver and spleen and no signs of abnormal erythropoiesis. The placenta showed considerable edema. No syphilitic changes were observed in the fetus or placenta. The signs of toxemia disappeared rapidly after delivery, the prompt regression of the edema being especially noticeable.

In 1942 and 1945 the patient had a normal delivery, each time of a healthy and normally developed child. On both occasions the course of pregnancy was normal, and no signs of erythroblastosis were noted in either child.

On admission to the hospital on January 16, 1948, the patient presented considerable edema of the face, abdomen and legs. During the fortnight prior to admission she had gained about 5 Kg. in weight. Urinalysis showed albuminuria of 1 per cent with no casts or erythrocytes. Blood pressure was 150/95. Ocular examination showed simple hypertensive changes in the fundi. Examination of the blood revealed: urea 30 mg., sugar 102 mg., uric acid 9 mg., total proteins 4.36 Gm., albumin 2.46 Gm. and globulin 1.9 Gm. per 100 ml., and the Wassermann and Kahn tests were negative. Tests showed the mother's blood to be B Rh₁ Hr-negative. The anti-A titration gave a titer of 1:64 by the ordinary agglutination

TABLE 1
BLOOD GROUPS AND RH TYPES OF PARENTS AND OFFSPRING AND OUTCOME OF PREGNANCIES

BLOOD OF	GROUP	Rh TYPE	Hr REACTION*	COURSE OF PREGNANCY
Patient.....	B	Rh ₁	Negative	
Husband.....	O	Rh ₁	Negative	
1st child.....	Hydrops fetalis			Toxemia
2nd child.....	B	Rh ₁	Negative	Normal
3rd child.....	B	Rh ₁	Negative	Normal
4th child.....	Hydrops fetalis			Toxemia

* This was performed with anti-Hr' serum. The other anti-Hr serums were not available.

technie as well as by the conglutination method. Tests revealed the husband's blood to be O Rh₁ Hr-negative. In view of this fact no anti-Rh or anti-Hr antibodies were expected in the patient. The examination of the patient's serum revealed no anti-Rh antibodies (Table 1). In order to exclude definitely the presence of any immune antibodies, a control test was made cross-matching the patient's serum with her husband's erythrocytes, and this also proved negative.

The uterus corresponded in its size to that of an eighth month pregnancy and fetal heart tones were audible. The patient was given the routine treatment for toxemia of pregnancy, viz., bed rest, salt-free diet, magnesium sulphate intramuscularly and sedatives. Her diuresis, which during the first days yielded from 300 to 500 cc. per day, slowly increased. On the eighth day after admission the patient noted that she did not feel fetal movements. In fact, on this day no fetal heart tones could be detected. Three days later the patient went into labor and delivered a macerated hydropic male fetus, weighing 2300 Gm. and an extremely hydropic placenta weighing 1200 Gm.

The postmortem examination of the fetus showed universal edema of all tissues with effusion into all serous cavities. The spleen and liver were not enlarged and no evidenece of erythroblastosis was present. The placenta showed a very edematous stroma with well preserved syncytium. No abnormal inerease in nueleated red blood cells was observed. Because of the strong hemolysis and maeeration no tests of the blood group or Rh type of the fetus could be made. Following the death of the fetus and delivery there was a rapid regres-

sion of all signs of toxemia. Blood proteins rapidly increased and normal values were found in the second week after delivery.

Anti-A titration in the patient and cross-matching with the husband's erythrocytes were performed on the 8th and 16th days after delivery (during the period of the expected rise in antibody titer in cases of erythroblastosis) and these tests did not show any evidence of immunization. No anti-Rh antibodies were found.

The blood typing of the patient's two living children are given in Table 1.

DISCUSSION

Generalized edema of the fetus (hydrops fetalis) occupies a special place among the three clinical pictures of hemolytic disease of the newborn, *i.e.*, hemolytic jaundice, congenital anemia and hydrops fetalis. In the first two, the clinical picture is the direct consequence of the hemolysis of fetal erythrocytes brought about by the maternal immune antibodies. Hydrops fetalis, on the other hand, is considered to follow a lowered blood protein level resulting from liver damage secondary to the hemolytic process in the child.² In such cases profound liver damage is usually found.⁷ It would, therefore, seem possible that any condition, other than hemolysis, leading to hypoproteinemia in the fetus might also cause hydrops fetalis.

In the case here reported we are able to exclude hemolytic disease of the newborn as the cause of the recurring fetal hydrops. In fact, none of the conditions required for the diagnosis of erythroblastosis fetalis were present in our case. These conditions are: 1. antigenic incompatibility between mother and fetus; 2. presence of immune antibodies in the maternal blood; 3. evidence of erythroblastosis in the newborn: anemia or jaundice, splenomegaly, hepatomegaly and an increased number of immature red blood cells in the fetal blood or placenta.

The fact that hydrops fetalis may occur unassociated with erythroblastosis permits us to assume that at least two forms of hydrops fetalis may exist: one form, an erythroblastotic hydrops, in which the above mentioned conditions are present, and a second form, a non-erythroblastotic hydrops, related to other still unknown causes. Since the true nature of this second form of hydrops fetalis is as yet unknown, it may be classified only after the exclusion of the presence of hemolytic disease of the newborn resulting from antigenic incompatibility between mother and fetus.

In the few cases of hydrops fetalis reported in the literature in which no Rh incompatibility could be detected, it has been emphasized that this condition may occur in primiparas and does not recur in subsequent pregnancies.⁶ Our observation of recurring hydrops fetalis is, to our knowledge, the first instance of this nature reported in the literature.

Of special interest is the fact that the course of pregnancy which terminated with the delivery of a hydropic fetus was complicated by a severe pre-eclamptic toxemia which was characterized chiefly by considerable maternal edema with marked hypoproteinemia. On the other hand, the two pregnancies that terminated in the delivery of normal children had quite a normal course. This observation points to the possible role of toxemia of pregnancy in the etiology of hydrops fetalis.

SUMMARY

A case of recurrent hydrops fetalis not caused by hemolytic disease of the newborn is reported. The patient delivered two hydropic infants in her first and fourth deliveries, while her other two pregnancies terminated in the delivery of normal children. Antigenic incompatibility between mother and fetus could be excluded. No maternal immunization or signs of fetal erythroblastosis were found. Both pregnancies which terminated in the delivery of hydropic infants were associated with a severe toxemia of pregnancy, while the other pregnancies had a normal course.

The fact that hydrops fetalis occurred unassociated with erythroblastosis leads to the assumption that two forms of hydrops fetalis exist: an erythroblastotic and a non-erythroblastotic form. This case indicates that the non-erythroblastotic hydrops fetalis may be related to toxemia of pregnancy.

Acknowledgments. We are indebted to the Bacteriologic and Serologic Laboratory and the Pathologic Institute of the Hadassah University Hospital for the serologic and pathologic studies in this case.

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DIFFERENTIAL DIAGNOSIS OF RHEUMATOID ARTHRITIS BY BIOPSY OF MUSCLE*

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Nodular inflammatory lesions in skeletal muscles of patients with rheumatoid arthritis have been reported in varying frequency. Similar lesions have also been described in a small group of other diseases. The purposes of this paper are first, to determine from our biopsy material the incidence of cellular infiltrations in skeletal muscles in diseases other than rheumatoid arthritis and second, to establish, if possible, further evidence for the histologically specific nature of the polymyositic nodules in rheumatoid arthritis.

Curtis and Pollard⁵ in 1940 described cellular infiltrations in the subcutaneous tissues and skeletal muscles in 8 of a group of 11 patients: 3 with Felty's syndrome, 4 with rheumatoid arthritis, splenomegaly and leukocytosis and 4 with rheumatoid arthritis without splenomegaly and leukopenia. In the opinion of the authors, the perivascular lymphocytic infiltration was an indication of either a close relationship between, or the identity of, Felty's syndrome and rheumatoid arthritis. Freund and co-workers,^{7a,b} guided by their previous findings in peripheral nerves, examined sections of skeletal muscles of patients with rheumatoid arthritis. In 1945 and 1946, Steiner and co-workers^{8, 13} reported having found typical inflammatory nodules in biopsies or in sections of skeletal muscles taken at autopsy from 24 patients with this disease. These cellular infiltrations were present in the endomysium and perimysium, rarely in the epimysium, and were composed of lymphocytes and plasma cells with very occasional mast cells and rare neutrophils. There were very small amounts of collagenous tissue within the nodules and no reticulin networks. The adjacent muscle fibers had undergone various types of degenerative change which they interpreted as secondary to the presence of the inflammatory reaction. The control material reported by them consisted of sections of skeletal muscles in 196 routine autopsies, and of biopsies from 7 patients in whom skeletal muscle lesions were to be expected. No similar cellular accumulations were found in any of these controls. Clawson³ confirmed these findings, stating that he had found similar infiltrations in biopsies of skeletal muscles of more than one-half of approximately 70 patients with rheumatoid arthritis. He also reported having seen lesions of a similar nature in a few patients with acute rheumatic fever and in one with a healed rheumatic valvular deformity. Black-Schaffer² mentioned similar lesions in one patient with dermatomyositis and one with scleroderma. Gibson, Kersley and Desmarais¹⁰ found cellular infiltrations in the skeletal muscles taken for biopsy in all of 11 patients with rheumatoid arthritis. deForest, Bunting and Kenney⁶ examined skeletal muscles taken for biopsy from 16 patients with a clinical

* Received for publication, July 14, 1948.

diagnosis of rheumatoid arthritis. Typical nodular infiltrations were seen in 13 of the 16. Of the three patients in whom no infiltrations were found, two had Marie-Strümpell's disease and one possibly had disseminated lupus erythematosus. Similar lesions were also found in two of their four patients with nonspecific infectious arthritis and in one of four patients with osteoarthritis. No lesions were seen in 5 patients with rheumatic fever, in one with gonorrheal arthritis and in 17 autopsied subjects. Bauer,¹ in discussing this paper, mentioned that Morrison had found similar lesions in one case each of disseminated lupus erythematosus and dermatomyositis. Freyberg⁹ states that he had had two patients in whom the diagnosis of rheumatoid arthritis had been made by muscle biopsy several months before the clinical evidence permitted such a diagnosis. Clawson, Noble and Lufkin^{4a} reported nodular inflammatory lesions in the skeletal muscles in 26.2 per cent of 450 autopsies. These lesions were found most commonly in cases of acute rheumatic fever, rheumatoid arthritis and in older persons with various diseases. The muscles most frequently involved were the diaphragm and the intercostals. In their final report,^{4b} the sacrospinalis muscle was included as one of the muscles most frequently involved among a group of 8 different muscles studied. In their statistical analysis they made no effort to evaluate the different types of cellular infiltrations which they described. The frequency of trichinosis among their cases was neither mentioned nor considered. Finally, as stated by them, rheumatoid arthritis is a common condition in Minnesota and may have been present to some extent without having been mentioned in the histories. Morrison and co-workers¹² found cellular infiltrations in the muscles of 8 of 14 patients with rheumatoid arthritis examined at autopsy, and also in patients with dermatomyositis, disseminated lupus erythematosus, scleroderma, and rheumatic heart disease without evidence of arthritis. Kersley and Desmarais¹¹ recently reported the presence of cellular infiltrations in muscle biopsies from 25 of 30 patients with rheumatoid arthritis. One of the 5 negative cases was found to be positive when a larger portion of muscle was later removed. In 65 patients suspected of having other diseases, all were negative except two who had rheumatoid arthritis clinically simulating gout. Included in the group without any infiltrations were 12 patients with spondylitis ankylopoietica, four with gonorrheal arthritis, two with Still's disease and four with osteoarthritis.

MATERIAL AND METHODS

All the muscle specimens concerned in this report were taken either for biopsy or from amputations. The quantity of muscle examined, except in the amputations, was never large. The tissues were routinely fixed in formalin and stained with hematoxylin and eosin. Multiple sections and special stains were done whenever it was thought that they were indicated. Each section was first examined without knowledge of the clinical diagnosis and later re-examined after the diagnosis was known. The material consisted of 126 controls and 27 patients with all stages of rheumatoid arthritis. All persons except one were adults. The cases of rheumatoid arthritis and the controls are classified in Table 1.

TABLE 1
DIFFERENTIAL DIAGNOSIS OF RHEUMATOID ARTHRITIS BY MUSCLE BIOPSY

TYPE OF LESION	NO. OF CASES	NO. WITH CELLULAR INFILTRATION	PER CENT
Rheumatoid arthritis.....	27	26	96.3
Normal controls.....	12	0	0.
Atherosclerosis with gangrene.....	70	18	25.3
Diseases involving skeletal muscle			
a. Disseminated lupus erythematosus.....	16	7	43.7
b. Thromboangiitis obliterans.....	4	4	100.
c. Dermatomyositis.....	4	4	100.
d. Trichinosis.....	4	4	100.
e. Scleroderma.....	4	0	0.
f. Miscellaneous diseases			
1. Chronic suppurative myositis.....	1	1	100.
2. Myasthenia gravis.....	1	0	0.
3. Gas gangrene with secondary infection....	1	1	100.
4. Periarthritis nodosa.....	1	1	100.
5. Nonspecific granuloma (sarcoidosis?).....	1	1	100.
6. Arachnodactylia.....	1	0	0.
Polyarthritis, not rheumatoid			
a. Infectious.....	2	0	0.
b. Rheumatic.....	1	0	0.
c. Spondylitis.....	2	0	0.
d. Nonspecific.....	1	0	0.
	126	41	32.5

TABLE 2
DIFFERENTIAL DIAGNOSTIC FEATURES OF INFILTRATIONS IN SKELETAL MUSCLE

DISEASE	CELL TYPES	DEMARCATON	LOCALIZATION	CONNECTIVE TISSUE STROMA
Rheumatoid arthritis	Lymphocytes and plasma cells	Sharp, nodular	Endo- and perimysial, rarely epimysial	Scanty
Disseminated lupus erythematosus	Lymphocytes, neutrophils, plasma cells	Sharp, nodular	Endo-, peri- and epimysial	Abundant, with fat cells
Thromboangiitis obliterans	Neutrophils, eosinophils, lymphocytes, macrophages, plasma cells	Poor	In and around blood vessels and nerves	Tendency to fibrosis in later stages
Dermatomyositis	Lymphocytes, neutrophils, plasma cells, eosinophils	Poor, not nodular	Endo-, peri- and epimysial and surrounding tissues	
Trichinosis	Eosinophils, neutrophils, lymphocytes	Sharp	About the parasite	Fibrosis and hyalinization, late

In Table 2 several types of cellular muscular infiltrations are distinguished according to their histologic characteristics.

DISCUSSION

Sections from the gastrocnemius and deltoid muscles and occasionally from other skeletal muscles were studied in 27 patients with clinically typical *rheumatoid arthritis*. These included very early cases as well as those in which the disease was far advanced. No special effort was made to remove muscle in the neighborhood of subcutaneous nodules or involved large joints. Nodular infiltrations (Fig. 1a) were composed most commonly of an inner group of lymphocytes with an outer layer of irregularly disposed plasma cells. In a few cases some of the larger nodules contained an inner core of cells of epithelioid appearance (Fig. 1b). Such nodules were found in 26 of the 27 patients, were sharply circumscribed and were always located in the endomysium and/or the perimysium, and rarely in the epimysium. A small blood vessel was often seen at the periphery and very little connective tissue was present within the nodule. The adjacent muscle fibers revealed varying degrees of degenerative change which was secondary to the inflammatory reaction. The single case in which no nodular infiltration was found was that of a 74 year old woman who had had arthritis for over thirty years.

For *normal controls*, skeletal muscles were taken from 12 patients, one of whom had had a traumatic amputation. No cellular infiltration was found in any of the muscle sections.

The skeletal muscles of 71 lower extremities amputated because of *atherosclerosis with gangrene* comprised the second group of controls. This material came from 70 different patients, 20 of whom also had diabetes mellitus. Cellular accumulations of all types were found in the muscles of 18 patients, including 12 who were not diabetic and 6 who were diabetic. The infiltrates were composed either of neutrophils only or of a diffuse mixture of neutrophils and lymphocytes and sometimes a few scattered mononuclear phagocytes, eosinophils and plasma cells. No purely lymphocytic and plasma cell infiltrations were seen. In general, the infiltrations were poorly circumscribed and appeared to be extending into the muscles. Abscesses were occasionally found.

In the third control group, there were 38 cases with diseases in which cellular infiltrations in the skeletal muscles either have been described or may be expected to occur. There were 16 patients with disseminated lupus erythematosus, 4 with thromboangiitis obliterans, 4 with dermatomyositis, 4 with trichinosis,

FIG. 1a. G. M. Rheumatoid arthritis. Skeletal muscle biopsy. H and E. $\times 150$.

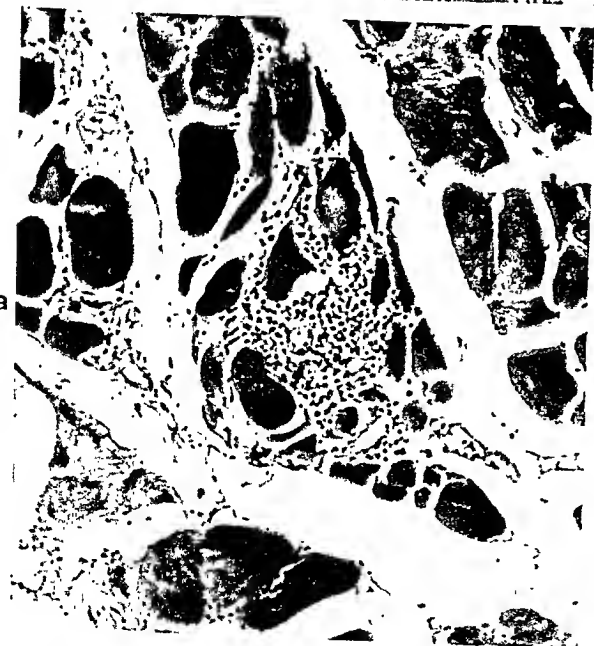
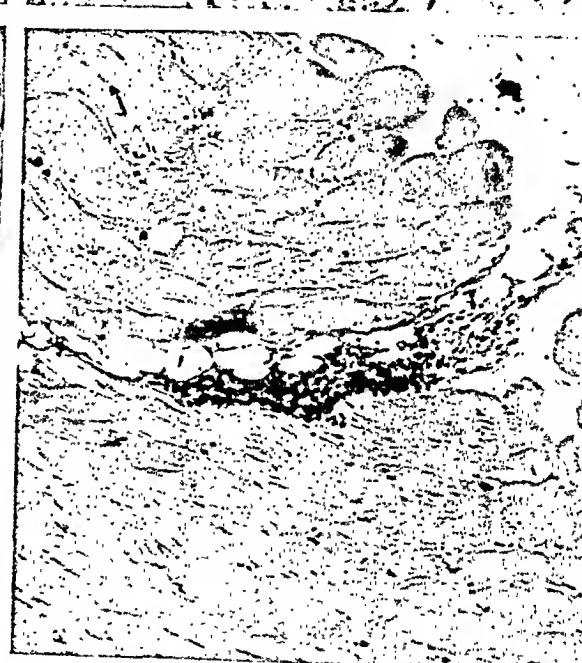
FIG. 1b. G. M. Rheumatoid arthritis. Skeletal muscle biopsy. H and E. $\times 150$.

FIG. 2. C. S. Disseminated lupus erythematosus. Skeletal muscle biopsy. H and E. $\times 140$.

FIG. 3. S. V. Disseminated lupus erythematosus. Skeletal muscle biopsy. H and E. $\times 115$.

FIG. 4a. M. W. Disseminated lupus erythematosus. Skeletal muscle biopsy. H and E. $\times 140$.

FIG. 4b. M. W. Disseminated lupus erythematosus. Skeletal muscle biopsy. H and E. $\times 140$.



FIGS. 1a-4b

4 with scleroderma and one each with chronic suppurative myositis, myasthenia gravis, gas gangrene with secondary infection, periarteritis nodosa, nonspecific granuloma (sarcoidosis?) and arachnodactylia.

Cellular accumulations were discovered in sections from only 7 of the 16 patients with *disseminated lupus erythematosus*. The incidence of these infiltrations was thus lower than in our patients with rheumatoid arthritis. The infiltrations in 5 of the 7 patients were composed of neutrophils and lymphocytes and an occasional plasma cell. Furthermore, the nodules contained an abundant connective tissue stroma and fat cells at the periphery of the nodules (Fig. 2). The infiltrates of the remaining two patients were composed only of lymphocytes with some peripheral plasma cells, were present in the endomysium and perimysium, and were well circumscribed. However, in one case (Fig. 3) the small nodule was surrounded by many fat cells and likewise contained more collagenous tissue than was present in the nodules of rheumatoid arthritis. The nodules in the muscle of the one remaining patient* (Figs. 4a, 4b) could not be differentiated from those seen in rheumatoid arthritis. It is of interest to note that in all of the patients in whom a cellular infiltration was found, joint pains were a prominent symptom.

The infiltrations in the four patients with *thromboangiitis obliterans* were composed of neutrophils, mononuclear macrophages, lymphocytes, eosinophils and plasma cells. These cells were present within the blood vessels walls and surrounded the blood vessels and the accompanying nerves (Figs. 5a, 5b, 6). These infiltrations were frequently at a distance from the muscles and, in general, were poorly circumscribed.

Sections from the four patients with *dermatomyositis* contained very poorly demarcated infiltrations composed of lymphocytes and neutrophils with some eosinophils and plasma cells.

Trichinosis infection of skeletal muscles occasionally offered some difficulty in sections containing only the peripheral zones of the nodular infiltrations. Studies of multiple sections, however, in each case revealed the presence of a parasite and/or many eosinophils and neutrophils.

Cellular infiltrations were not seen in any of the sections of muscle from the four patients with *scleroderma* although there were lymphocytic cuffings about some of the vessels in the subcutis.

The *miscellaneous diseases* of muscles other than mentioned previously formed the next group of controls. In the four cases in which infiltrations were found, there was no similarity to those seen in rheumatoid arthritis.

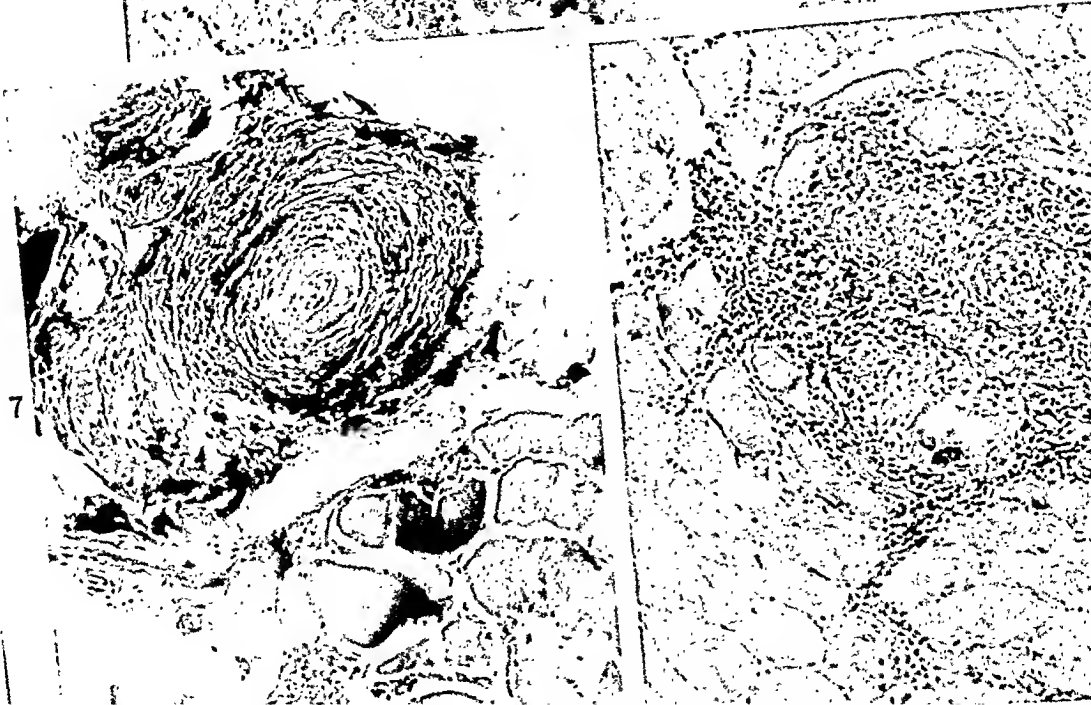
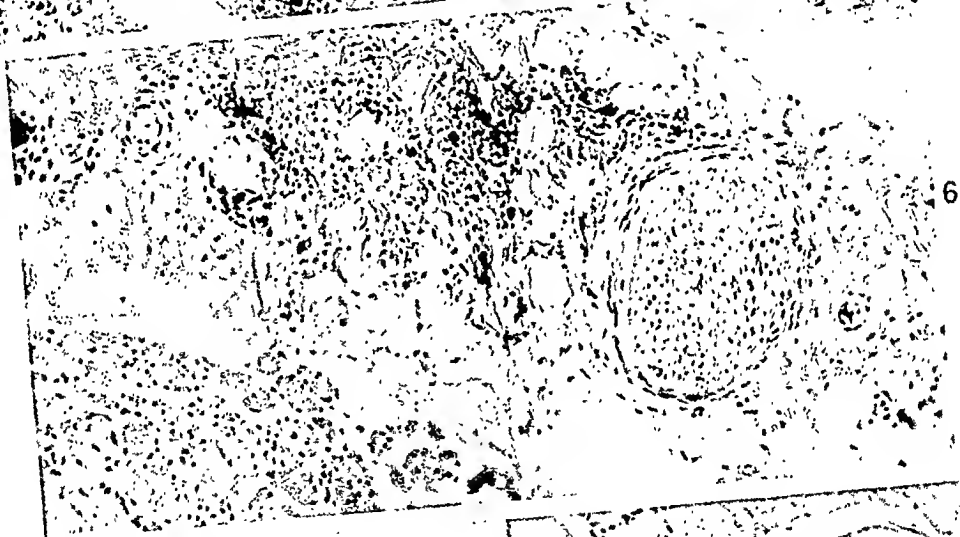
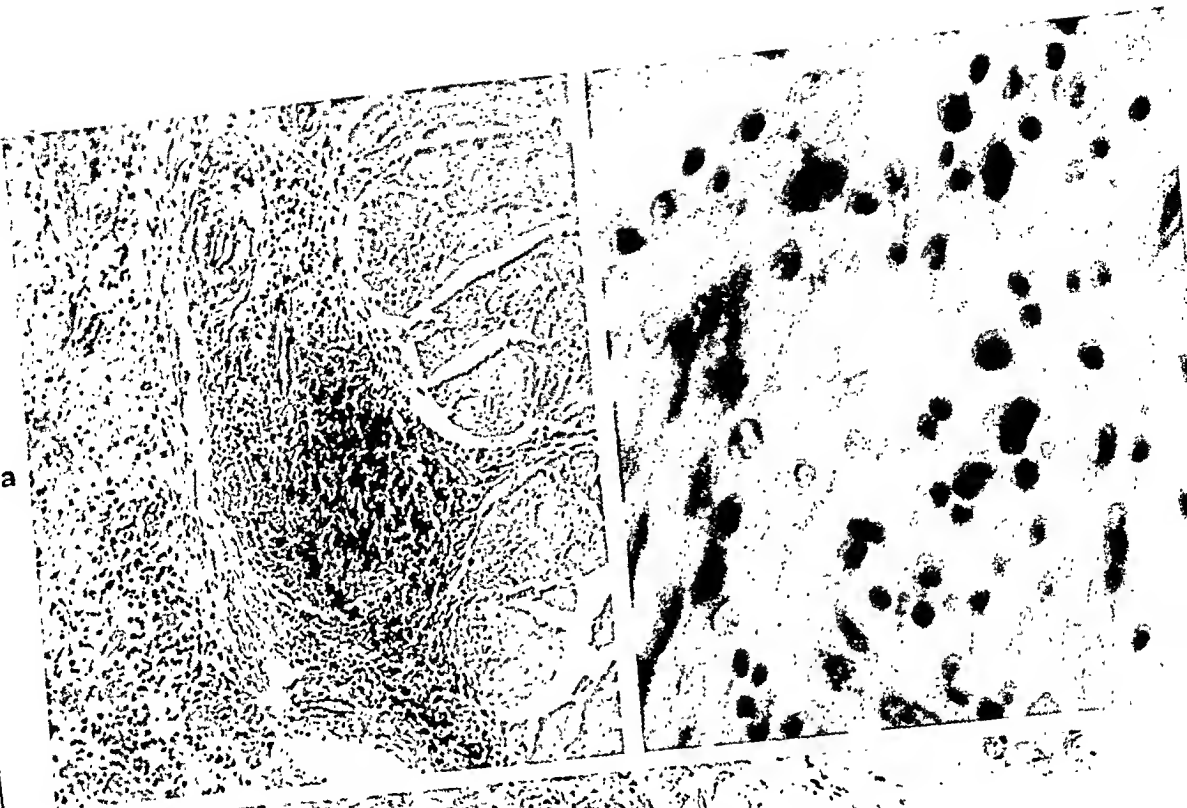
* This patient later died. In the skeletal muscles taken at autopsy, the differences between the nodules seen in patients with disseminated lupus erythematosus and in those from patients with rheumatoid arthritis were clearly demonstrated.

FIG. 5a. L. K. Thromboangiitis obliterans. Skeletal muscle biopsy. H and E. $\times 115$.
FIG. 5b. L. K. Thromboangiitis obliterans. Skeletal muscle biopsy. H and E. $\times 500$.

FIG. 6. R. L. Thromboangiitis obliterans. Skeletal muscle biopsy. H and E. $\times 140$.

FIG. 7. G. R. Polyarteritis nodosa. Skeletal muscle biopsy. van Gieson. $\times 140$.

FIG. 8. M. B. Nonspecific granuloma (sarcoidosis?) Skeletal muscle biopsy. van Gieson. $\times 140$.



The sections from the patient with chronic suppurative myositis contained chronic abscesses. The muscles from the patient with gas gangrene with secondary infection contained diffuse infiltrations composed of neutrophils and lymphocytes. The infiltrations in the subject with periarteritis nodosa were situated in the greatly thickened and hyalinized walls of the arterioles and contained neutrophils and lymphocytes (Fig. 7). The nodular infiltration in the muscle of the fourth patient represented a typical granulomatous inflammation characterized by epithelioid tubercles (Fig. 8). The clinical condition was suggestive of sarcoidosis.

The fifth group of controls were 16 patients with *polyarthritis not of a rheumatoid nature*. No cellular infiltrations were found in any of the sections of muscle.

SUMMARY

1. Typical nodular cellular infiltrations were found in the skeletal muscles in 26 of 27 patients in all stages of clinically typical rheumatoid arthritis. These nodules were present in muscles remote from subcutaneous nodules and involved joints.

2. The control material was composed of muscles from 126 persons, as follows: 12 normal persons; 70 patients with atherosclerosis and gangrene, 20 of whom also had diabetes mellitus; 16 patients with disseminated lupus erythematosus; 4 with thromboangiitis obliterans; 4 with dermatomyositis; 4 with trichinosis; 4 with scleroderma; 6 with miscellaneous diseases of muscle; and 6 with non-rheumatoid polyarthritis (including only one with rheumatic fever).

3. The histopathologic differentiation between rheumatoid arthritis and the other diseases studied was made without difficulty in 125 of the 126 controls.

4. In 6 of 7 patients with disseminated lupus erythematosus in whom cellular infiltrations were present, the muscle lesions were differentiated. In the seventh patient the lesions were so similar that differential diagnosis was not possible.

5. Of other diseases in which cellular infiltrations were found, the histopathologic characteristics of the infiltrates were such that the differential diagnosis could be made without difficulty.

CONCLUSIONS

1. Typical nodular cellular infiltrations in skeletal muscles occur in a high percentage of patients in all stages of rheumatoid arthritis.

2. Cellular infiltrations, sometimes seen in the nonrheumatoid diseases studied, can be histologically distinguished in almost every instance.

Acknowledgments. We wish to express our appreciation to Dr. O. A. Brines, Dr. H. A. Freund and Dr. S. E. Gould for making available the pathologic material.

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CARDIOLIPIN ANTIGEN IN THE KLINE TEST FOR SYPHILIS*

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The literature on the use of cardiolipin for serologic testing in syphilis is in process of rapid accretion. Cardiolipin, the active antigenic component of beef heart lipoid, was isolated by Pangborn¹³ in 1941, and she recently reported¹⁴ the calculated chemical formula of its sodium salt as $C_{120}H_{208}O_{24}P_3Na_3$.

In a previous report¹⁰ we presented data on highly satisfactory results obtained with the Kline cardiolipin slide test in the serodiagnosis of syphilis. It was found desirable to modify Kline's recommended cardiolipin-lecithin ratio in order to obtain results of optimal specificity. This modified procedure has since been applied in routine testing for syphilis. A total of 11,104 blood samples has been tested to date. The present paper gives an analysis of the results obtained with 4718 serums tested with the modified ratio. Further data are given on the ratio of cardiolipin and lecithin optimal for use in the Kline slide test.

Special reference will be made here only to articles on cardiolipin testing which appeared subsequent to the submission of our first report. All these recent experiences were favorably reported and proved a very high sensitivity level for cardiolipin antigen. The question of increased specificity has not been as clearly demonstrated (Mahoney¹²), except in the special case of malaria.

Kline, on the basis of very extensive testing, reports most enthusiastically on cardiolipin as the "apparent end of search for the active antigenic factor that combines with syphilis reagin".⁸ "The excellent results obtained with cardiolipin antigen and the great simplicity of the slide flocculation technic recommend the two as a base for a single standard test for syphilis worthy of universal adoption."⁷

Levine, Kline and Suessenguth¹¹ reported a clinical evaluation of 27,103 comparative slide tests with cardiolipin-lecithin, Kline diagnostic and Kline exclusion antigens. They concluded that the cardiolipin-lecithin antigen showed both the greatest specificity and greatest sensitivity and have adopted it as standard antigen for the Kline test. They obtained only 0.228 per cent of combined false-positive and doubtful reactions with nonsyphilitic serums and commented that "no greater specificity in a test for syphilis is likely to be achieved". In syphilitic serums only two negative cardiolipin results were obtained in 4581 tests.

Andujar, Anderson and Mazurek¹ compared results obtained with standard and cardiolipin antigen in the Kline slide test and in the Harris and Portnoy⁴ modification of the Kolmer complement-fixation test. They presented a break-

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down of data for the different clinical stages of syphilis and concluded that the cardiolipin antigen showed "generally superior specificity and generally very slightly increased sensitivity". They noted in many cases a persistent cardiolipin seropositivity years after clinical "cure" and negativity to other serum tests.

Shaw¹⁶ compared cardiolipin-lecithin antigen with Kline exclusion antigen in the Kline slide test and concluded that the former was both more sensitive and more specific. Shaw reported that cardiolipin is more sensitive than standard Kolmer antigen in the Kolmer complement-fixation test and is especially advantageous in diagnosing low reagin-titered icteric serums.

Two recent papers have appeared on the VDRL (Venereal Disease Research Laboratory) slide test. This procedure⁵ differs in the essential detail that cholesterol is added in solution to the cardiolipin-lecithin solution prior to emulsification in saline, while in the Kline technic the antigen emulsion is prepared by coating previously precipitated cholesterol crystals with the cardiolipin-lecithin. The VDRL antigen emulsion contains less than one-half the cholesterol concentration of the Kline antigen and, therefore, shows much fewer suspended particles.

Giordano, Culbertson and Higginbotham³ compared the VDRL test and Mazzini test on 24,085 serums and concluded that the former "was more sensitive in positive serums and about equally specific in negative serums". The authors noted a "slight increase of nonspecific doubtful reactions with cardiolipin" and suggested that these might be reduced by changing the cardiolipin-lecithin ratio. They also presented data showing the very close agreement of parallel tests with different cardiolipin lots.

Widelock¹⁸ compared results obtained with VDRL slide, Mazzini slide, Kahn and Kolmer tests on a group of 52,372 unselected routine serums and noted that the Mazzini gave a higher percentage of "reactors" than did the VDRL test.

In our previous report,¹⁰ when the cardiolipin-lecithin was used in the 1:10 ratio recommended by Kline, a very high sensitivity level was shown, but the specificity results were significantly lower than with the standard Kahn. Comparative tests on 155 serums from cases with confirmed histories of syphilis, including old treated cases, gave sensitivity values as follows: cardiolipin 96.8 per cent, Kolmer 90.3 per cent, Kahn 87.7 per cent. Tests on 3652 serums from presumably nonsyphilitic persons gave 1.16 per cent positive and 1.84 per cent doubtful cardiolipin reactions. The standard Kahn test on these serums showed 0.58 per cent positive and 1.07 per cent doubtful reactions. It was, therefore, considered desirable to adjust downward the cardiolipin-lecithin antigen reactivity in order to decrease the percentage of nonspecific reactions. It was first necessary to determine to what limit the reactivity might be reduced without sacrificing high sensitivity in syphilitic serums.

It has been repeatedly reported that the cardiolipin-lecithin antigen may be adjusted to any desired sensitivity level within limits, merely by altering the ratio of cardiolipin and lecithin. The sensitivity increases with increasing amounts of lecithin. The optimal amount and proportion of antigen components have been

variously reported for different cardiolipin technics. For the Kline slide test, Kline⁶ originally recommended a cardiolipin-lecithin ratio of 1:9.4 to 1:10.6. Klein and Leiby¹⁰ recommended a 1:8 ratio. Levine, Kline and Suessenguth¹¹ used a ratio of 1:9 to 1:10. The LaMotte Chemical Company now markets a cardiolipin-lecithin mixture standardized by Dr. Kline. Kline writes in a recent article⁹ "further studies should establish the optimal quantity and proportion of cardiolipin and of lecithin to use". Andujar, Anderson and Mazurek¹ used Kline cardiolipin diagnostic antigen in 1:9 ratio and Kline cardiolipin exclusion antigen in 1:8 to 1:11.4 ratios. They reported that the exclusion antigens made with amounts of lecithin less than in the 1:8 ratio were "too undersensitive to be of any value". Rein and Bossak¹⁵ used a technic similar to the Kline cardiolipin exclusion test, except for heating the antigen, and obtained best results with a 1:6.5 ratio.

More data are required on parallel comparative testing with different cardiolipin-lecithin ratios. Definitive establishment of the optimal ratio is basic to development of a standardized test. Because of the many uncontrollable variables involved in serodiagnostic testing, it was considered that the results of tests of a large series of individual serums at all reagin levels might enable us to draw conclusions that would be more valid than those based on the results of tests of a few pooled, diluted serums.

PROCEDURE

The method of preparation of cardiolipin antigen emulsion has already been reported in detail (Klein and Leiby¹⁰). The technic outlined by Kline⁶ was followed with modification of the cardiolipin-lecithin ratio, and all solutions were used in one-half amounts. Centrifugation of the antigen emulsion was not resorted to, since the objective was to lessen, not increase, antigen reactivity.

The recommended 1:8 cardiolipin-lecithin antigen had the following composition:

- a) 0.43 ml. of double distilled water
- b) 0.5 ml. of 1 per cent cholesterol in alcoholic solution ("cholesterol, Pfanstiehl, ash-free, precipitated from alcohol, for Kline test").
- c) 0.13 ml. of cardiolipin-lecithin in alcohol, made by mixing 1 volume of 0.2 per cent cardiolipin (Lederle) and 1.6 volumes of 1 per cent lecithin (Lederle). This amount of the mixed solution contains 0.1 mg. cardiolipin and 0.8 mg. lecithin.
- d) 1.22 ml. of 0.85 per cent sodium chloride (Merck, Reagent).

All serums submitted to the laboratory for routine syphilis serodiagnosis were tested by the standard Kahn and cardiolipin slide. Serums showing any trace of reaction in either test were further tested by the 2-tube Kolmer complement-fixation (standard procedure in Veterans Administration laboratories¹⁷). The test results were scored as positive, doubtful and negative according to the following scheme:

	NEGATIVE	DOUBTFUL	POSITIVE
Cardiolipin.....	0, \mp	\pm , 1	2, 3, 4
Kolmer.....	0	1 to 3	4*
Kahn.....	0, (1 to 1½ = trace†)	2 to 5½	6 to 12

* As recommended by Eagle,² this strict interpretation of "positive" was useful for purpose of classifying serums and did not enter into the determination of comparative sensitivity.

† This "trace" subgroup was separated only for comparison with the very weak (\pm) cardiolipin reactions. The Kahn "trace" group was considered "negative" for the purpose of calculating specificity and sensitivity.

Kahn positive serums were titrated by the quantitative Kahn procedure. Serums showing any reactivity in Kahn or cardiolipin were further tested against a battery of six cardiolipin antigens containing cardiolipin-*lecithin* ratios of 1:5, 1:6, 1:7, 1:8, 1:9, 1:10. In preparing the antigens, the amounts of all reagents were kept constant except that the alcoholic solution of *lecithin* was used in appropriately increasing amounts to give the desired ratios.

Results were evaluated by separate consideration of (1) positive syphilitic serums, (2) weakly positive syphilitic serums, and (3) nonsyphilitic serums.

Cardiolipin Battery Tests on Positive Syphilitic Serums

This group comprised 150 serums with confirmed clinical histories of syphilis, which showed complete (4+) complement-fixation in the Kolmer test. Quantitative Kahns in this group gave 47 per cent with 10 Kahn units or more. The results of the tests with the cardiolipin-*lecithin* battery are given in Figure 1. A continuous increase in sensitivity with increasing amounts of *lecithin* is seen between the limits tested. Cardiolipin-*lecithin* 1:5 gave 13 false-negative reactions and 27 doubtful reactions. At the other limit, the 1:10 ratio gave only one doubtful and 2 negative reactions (both negatives from the same case of cardiovascular lesions). Ratios of 1:5, 1:6, 1:7, all showed a considerable number of weak or negative reactions (37.2 per cent, 20.3 per cent, 10.9 per cent, respectively) and could be rejected as insufficiently sensitive. The 1:8 ratio proved effectively sensitive (98.7 per cent). In comparison to 1:10 antigen, it showed no additional negatives and only three additional doubtful reactions.

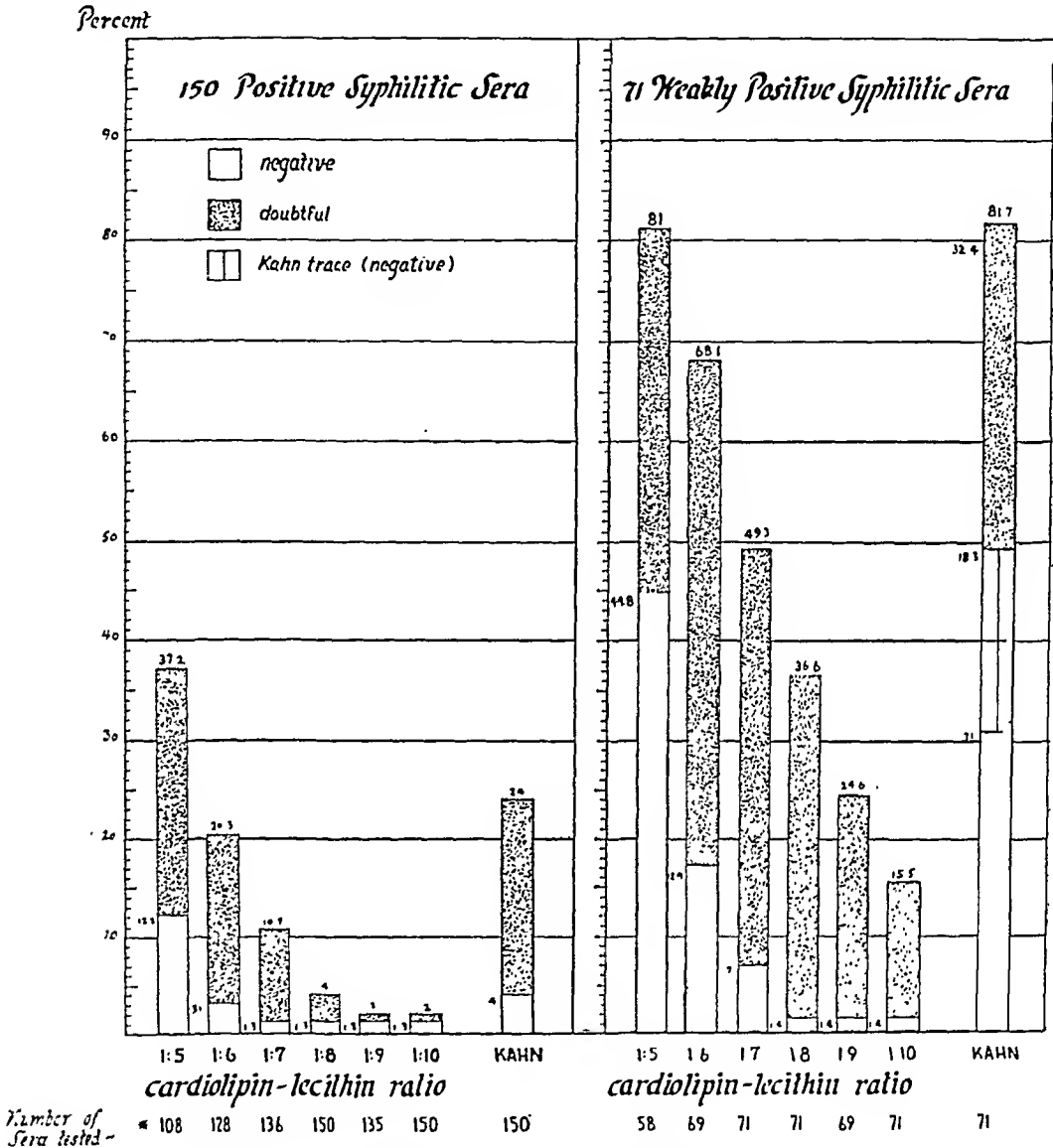
Kahn test results are included in Figure 1. Six false-negative reactions indicated a lesser sensitivity than all cardiolipin antigens other than the 1:5 ratio.

Cardiolipin Battery Tests on Weakly Positive Syphilitic Serums

This group comprised 71 serums from cases with confirmed clinical histories of syphilis, including old treated cases and latent cases, where the Kolmer test showed less than complete fixation or no fixation in the undiluted serum sample. The number of negative Kolmer reactions in this group was 27 (38 per cent). This group provided the most rigorous test of antigen sensitivity. It was im-

portant to determine that the cardiolipin-lecithin ratio selected for routine use prove adequately sensitive in this group of low reagin-titered serums.

The results of the battery test are recorded in Figure 1. Continuous increase in sensitivity with increasing amounts of lecithin is again seen throughout the



The volume of some serum specimens was insufficient for testing against all six cardiolipin antigens.

FIG. 1

Effect of Varying Cardiolipin-Lecithin Ratio on Per Cent of Negative and Doubtful Reactions with Syphilitic Serums.

range tested. The inadequate sensitivity of ratios 1:5, 1:6, 1:7, is even more apparent in this series. All showed high percentages of false-negative reactions—44.8, 17.4 and 7 per cent, respectively—and of combined doubtful and negative reactions—81, 68.1 and 49.3 per cent, respectively. Ratios 1:8, 1:9, 1:10 all showed only the same single negative reactions (a case of treated primary lues

with negative Kohner and Kahn serology) and were considered of satisfactory sensitivity (98.6 per cent). The 1:8 ratio showed more than twice as many doubtful reactions than did the 1:10 antigen.

The Kahn test showed 49 per cent negative reactions. Clearly, neither Kahn nor Kolmer tests approached the sensitivity level of the 1:8 cardiolipin-lecithin in this group of weakly positive syphilitic serums.

Cardiolipin Battery Tests on Nonsyphilitic Serums

Since we were primarily interested in reducing the number of nonspecific cardiolipin reactions, we selected for battery testing in this group those Kolmer negative serums with no history of syphilis which showed at least some trace of false reactions in routine Kahn or/and cardiolipin tests. Two hundred and eighty-three serums were available in this group after elimination of serums where clinical data indicated some history or suspicion of syphilis. Figure 2 shows a continuous increase in nonspecificity with increasing amounts of lecithin throughout the range of ratios tested. For reasons of serum selection, the level of nonspecificity was abnormally high throughout. Percentages of false-positive reactions obtained with cardiolipin-lecithin ratios of 1:5, 1:6, 1:7, 1:8, 1:9, 1:10 were, respectively, 1.6, 3.4, 6.0, 7.1, 10.4, 15.2. Percentages of combined positive and doubtful reactions were, in the same order: 11.0, 20.3, 28.6, 32.2, 38.5, 42.8.

Complete elimination of nonspecific reactions was not obtained even with those antigen ratios proven insufficiently sensitive on known syphilitic serums (ratios 1:5 to 1:7). The curve of increasing nonspecificity is seen to be gradual; there is no point of sudden increment in nonspecific reactions. Decision on the ratio optimal for routine diagnostic testing must, therefore, be based upon that ratio containing the least amount of lecithin compatible with high sensitivity in syphilitic serums.

The 1:8 ratio was shown in Figure 1 to be the lower limit of high sensitivity in tests on both strongly positive and weakly positive serums (showed no additional false negatives over the 1:10 ratio). The 1:8 ratio was, therefore, adopted for use in routine testing. Figure 2 shows that the 1:10 ratio gave 114 per cent more false-positive and 32 per cent more combined positive and doubtful nonspecific reactions than did the 1:8 ratio.

The 1:8 ratio showed a closer approach to the Kahn specificity obtained on this same group of serums selected for false precipitability than did the 1:10 ratio. The number of strongly positive nonspecific reactions with these antigens were: Kahn 3.6 per cent, 1:8 cardiolipin-lecithin 7.1 per cent, 1:10 cardiolipin-lecithin 15.2 per cent.

Specificity of 1:8 Cardiolipin-Lecithin With Nonsyphilitic Serum

A total of 4718 routine specimens was tested with cardiolipin-lecithin in 1:8 ratio. The serums were obtained from general hospital cases, blood donors and applicants for employment. Completely negative cardiolipin and Kahn results were obtained with 4302 serums which were, therefore, presumed to be nonsyphilitic. The remaining 416 serums were tested further by Kolmer comple-

ment-fixation test and were investigated for clinical and historical data for syphilis. Definite histories of syphilis were obtained in 205 cases. These are separately considered. Sixty serums were placed in the "undecided" category.

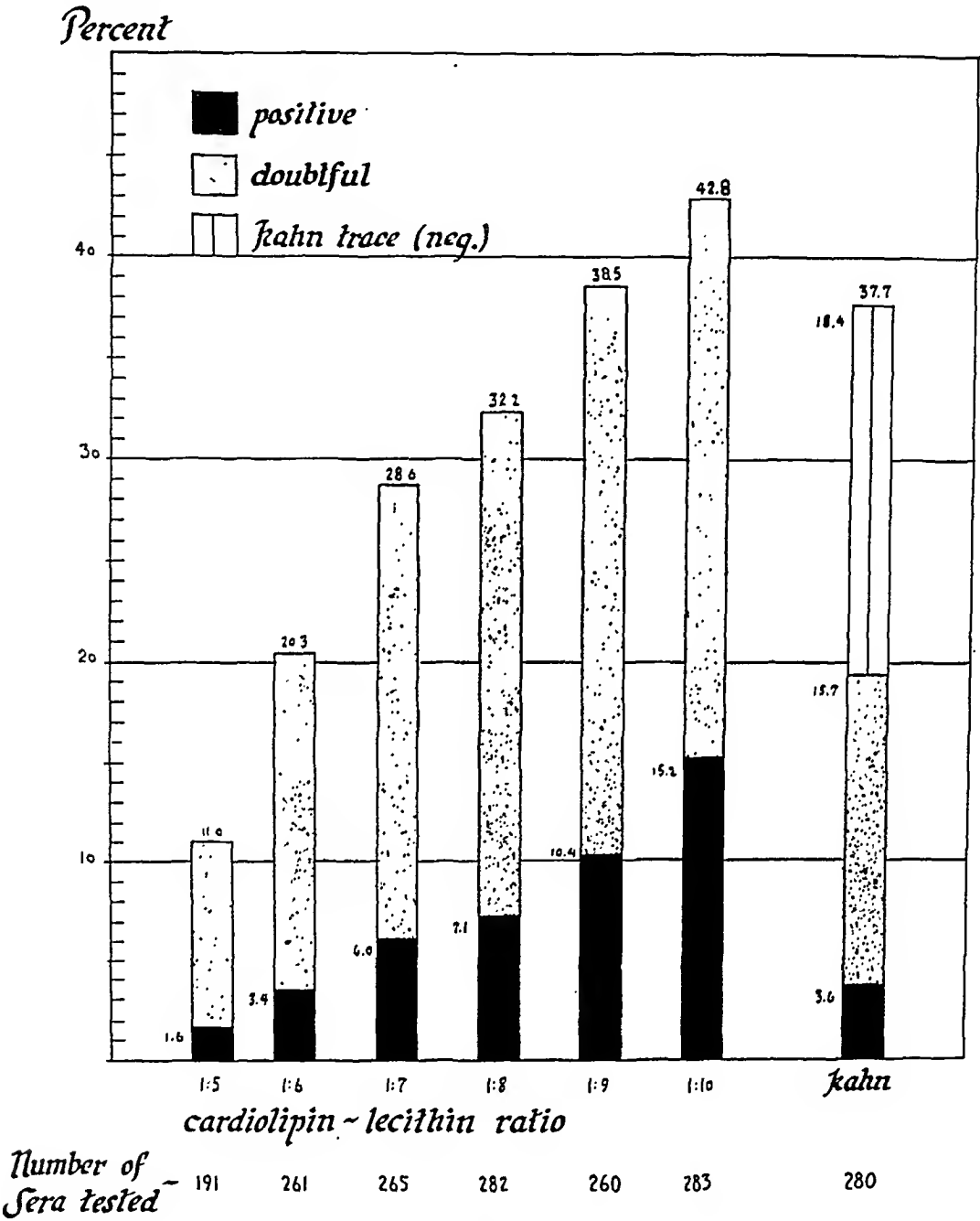


FIG. 2
Effect of Varying Cardiolipin-Lecithin Ratio on Per Cent of False-Positive Reactions with 283 Nonsyphilitic Serums.

These showed either a positive or weakly positive Kolmer test, or were still under clinical observation for possible lues. With the remaining 151 serums no significant data were available to justify exclusion from the "presumably non-

syphilitic group". These all showed negative Kolmer test and either had not been investigated for syphilis or had given only a negative history.

The cardiolipin and Kahn results obtained with the 4453 presumably nonsyphilitic serums are recorded in Figure 3. Cardiolipin-lecithin 1:8 showed only 0.29 per cent false-positive reactions. Subtracting also the 0.92 per cent of doubtful reactions gave a specificity of 98.8 per cent.

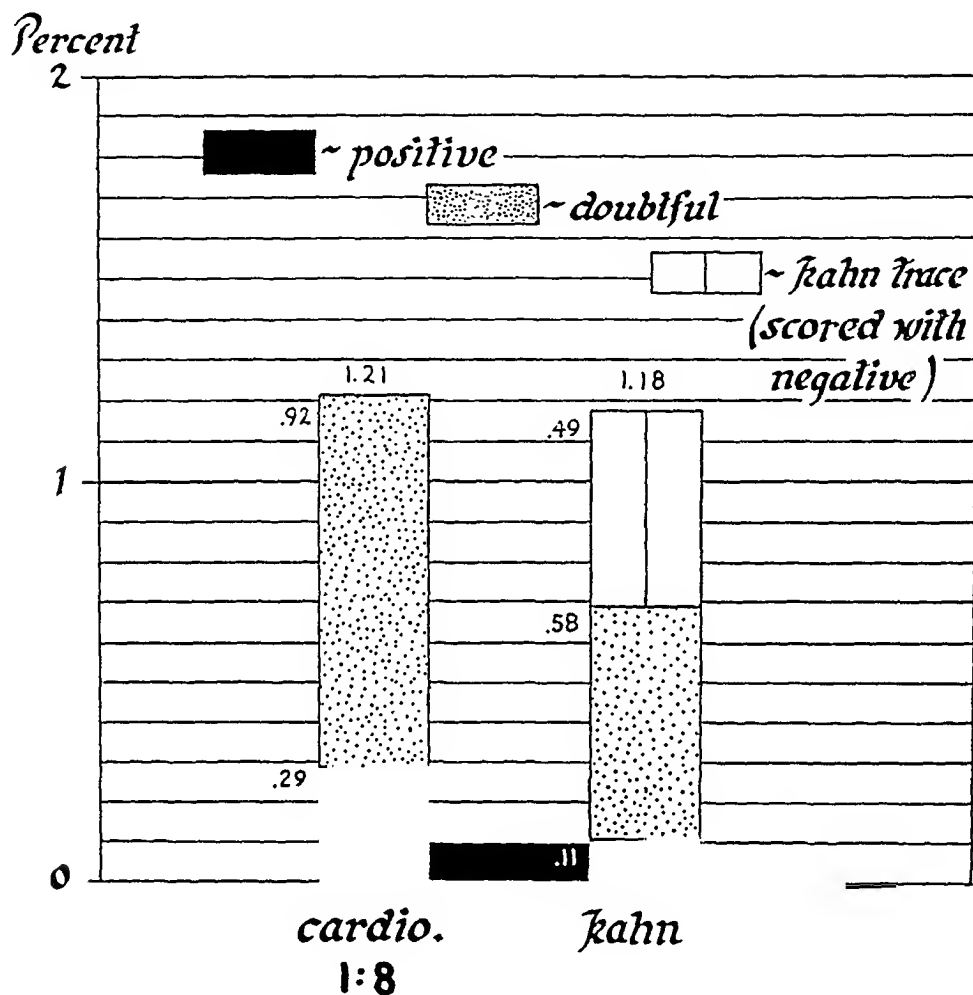


FIG. 3

False-Positive Reactions with Cardiolipin and Kahn Tests on 4453 Presumably Nonsyphilitic Serums.

The Kahn test showed a specificity of 99.3 per cent, a significant increase. In accordance with standard practice, the Kahn "trace" reactions (less than 011) were not subtracted in this computation of specificity. But since slight (\pm) reactions were subtracted from cardiolipin specificity, it seemed logical to include consideration of these borderline Kahn reactions in a comparison of the two tests. When this was done, the nonspecific reactions with the Kahn test became 1.18 per cent, almost identical to the 1.21 per cent obtained with cardiolipin.

These figures on nonspecific reactions are unavoidably exaggerated and represent maximal figures. They include a large percentage of serums for which clinical data were unavailable or insufficient to make for reasonable certainty of the absence of syphilitic history. This applies, for example, to 7 of the 13 cardio-

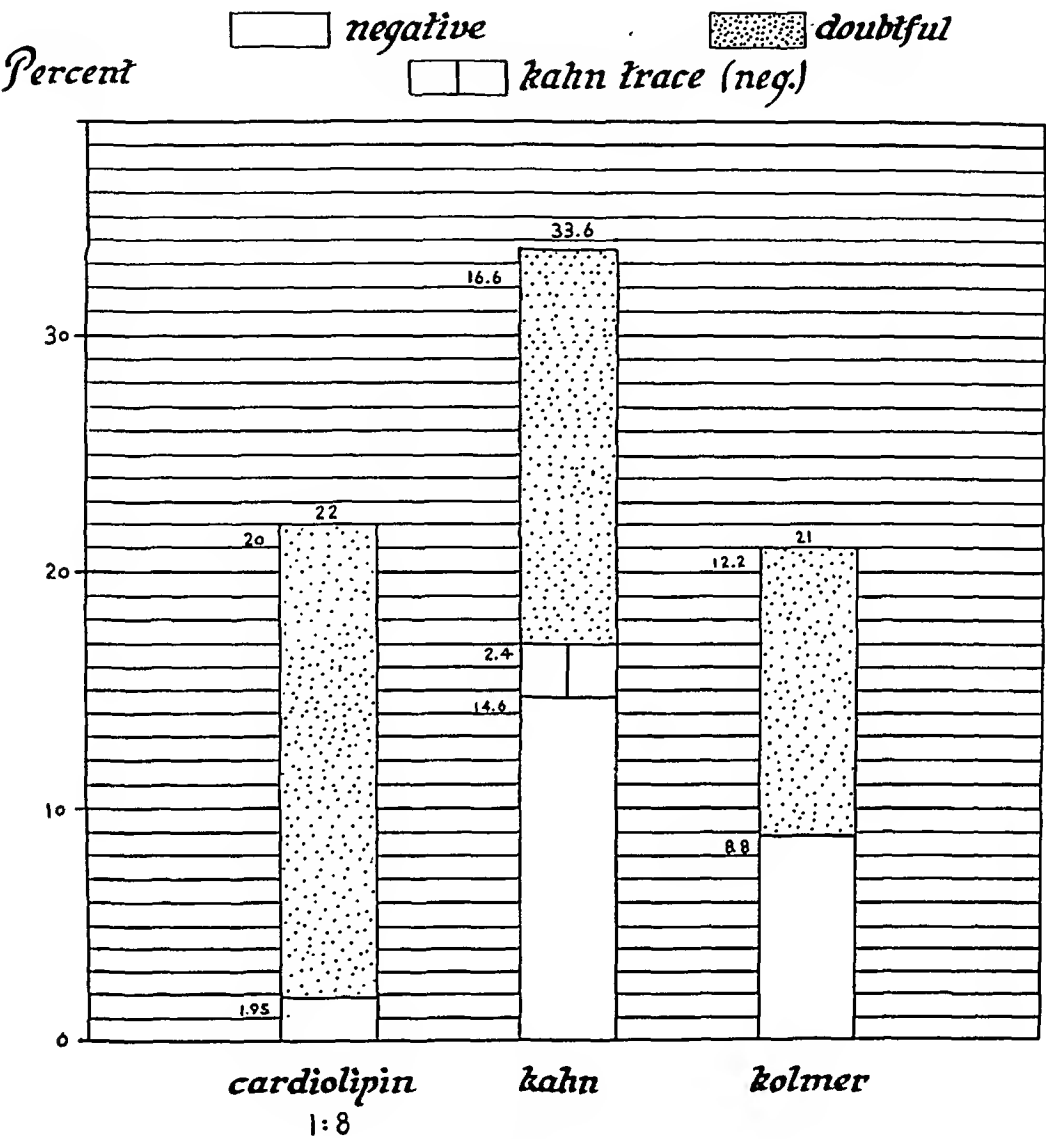


FIG. 4
Comparative Sensitivity of Cardiolipin, Kahn and Kolmer Tests on 205 Syphilitic Serums.

lipin “false positive” and to 33 of the 41 cardiolipin “false doubtfuls” No doubt a few of these do have a history of old treated syphilis despite their negative Kolmer test. On the other hand, elimination of all 40 of these serums with “no data” would clearly be unfair sampling. Efforts to elicit histories of syphilis are inaccurate at best. Therefore, with scanty data on histories of syphilis available in this presumed nonsyphilitic group, no attempt is made to arrive at a corrected minimal percentage of false cardiolipin reactions. The true percent-

age for this series must be somewhere below the maximal figure of 1.2 per cent, but exceeds the remarkably low figure of 0.228 per cent obtained by Kline.

Analysis of false cardiolipin reactions for case histories revealed no significant number in any particular disease category.

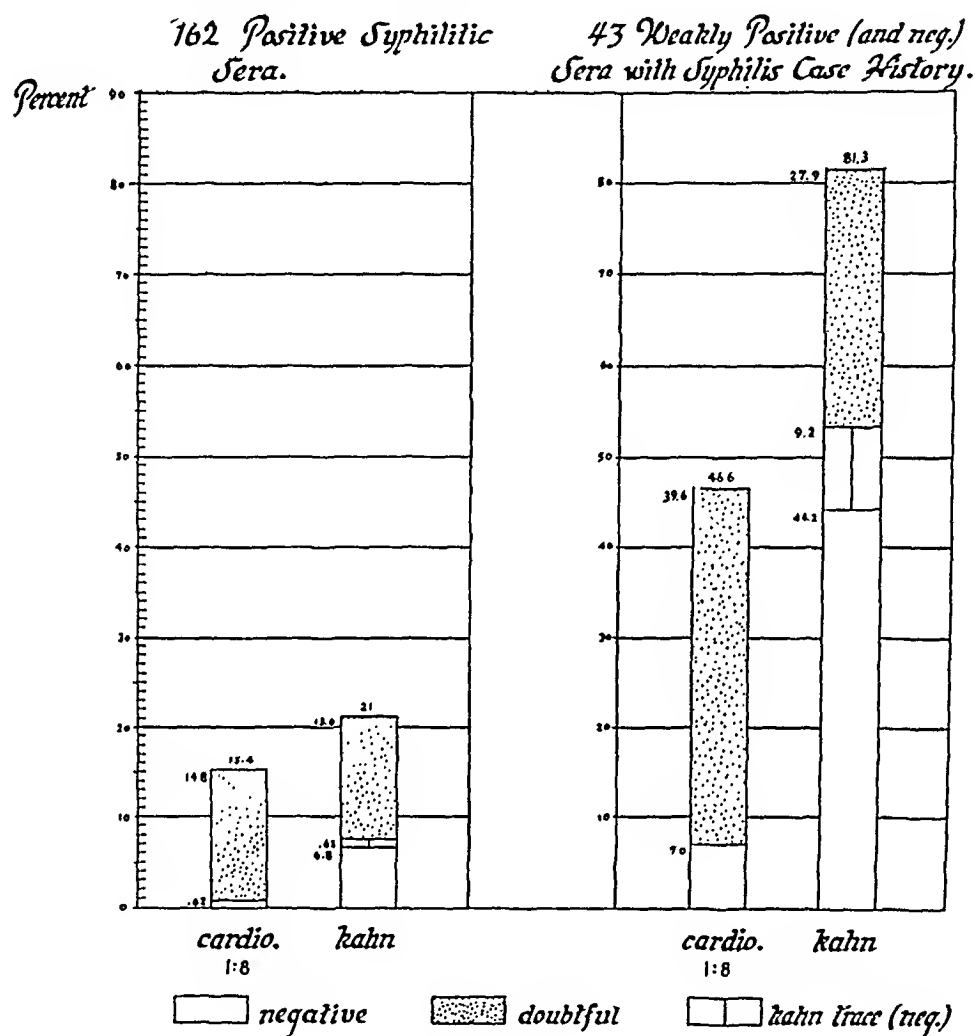


FIG. 5

Negative and Doubtful Reactions with Cardiolipin and Kahn Tests on 205 Syphilitic Serums.

Sensitivity of 1:8 Cardiolipin-Lecithin with Syphilitic Serums

A total of 205 serums was available from patients with definite clinical histories of syphilis. These included clinically active, latent and adequately treated patients. Comparative results obtained with the cardiolipin, Kolmer and Kahn tests are recorded in Figure 4. The number of negatives obtained in each test was: cardiolipin 4, Kolmer 18, Kahn 35. The comparative sensitivities were, therefore: cardiolipin 98 per cent, Kolmer 91.2 per cent, Kahn 83 per cent.

Cardiolipin-lecithin 1:8 is clearly the most efficient of the three antigens for screening purposes.

The four negative cardiolipin results included only one case of active clinical disease (cardiovascular lues, quantitative Kolmer 3 plus in 1:40 dilution, 1 plus anti-complementary in 1:8 dilution, greater than 160 Kahn units); the possibility of a negative cardiolipin prozone reaction was not tested here. The other three negatives were from old "probably adequately treated" cases with negative or doubtful Kahn and Kolmer tests.

For purpose of further analysis of cardiolipin and Kahn sensitivities, Figure 5 gives a breakdown of the results with these 205 known syphilitic serums into two categories on basis of the Kolmer reaction: (1) positive syphilitic serum, with complete (4+) fixation of complement, and (2) weakly positive syphilitic serums, with partial or no fixation of complement.

Positive syphilitic serums (162). Only one false-negative reaction was obtained with 1:8 cardiolipin-lecithin (sensitivity 99.4 per cent). The Kahn test gave 11 false-negative reactions and 1 trace reaction, a sensitivity of only 92.6 per cent. Both cardiolipin and Kahn gave essentially the same number of doubtful reactions, 14.8 and 13.6 per cent, respectively.

There were 20 "undecided" 4+ Kolmer serums which were not included in the above compilation because clinical or historical syphilis had not been established. Four negative cardiolipin tests were obtained in this group. Two were from a case definitively diagnosed as leprosy, one was a case of arthritis with no luetic investigation, or follow-up, the fourth was a case of virus pneumonia with a negative luetic history and a repeat specimen six days later which was Kolmer-negative.

Weakly positive syphilitic serums (43). These included 19 Kolmer-negative serums. The Kahn test showed 19 negative and 4 trace reactions (sensitivity 47 per cent), and was, therefore, entirely unreliable for detection of syphilis in this group of low-titered serums. Cardiolipin-lecithin 1:8 gave only 3 negative reactions, a sensitivity of 93 per cent. The superior sensitivity of cardiolipin was most pronounced in this group.

DISCUSSION

Decision on the cardiolipin-lecithin ratio to employ rests, of course, on the level of reactivity desired. However, maximum sensitivity can only be achieved at a sacrifice of specificity to a degree undesirable except for screening purposes. The 1:8 ratio adopted for routine diagnostic testing was necessarily a mean, selected on basis of both satisfactory specificity and high sensitivity. In parallel tests it showed a lesser reactivity than did the 1:10 ratio in that the percentage of weakly positive reactions with syphilitic serums was significantly increased. However, since no additional false-negative reactions were obtained in either low or high-titered serums, the sensitivity values were identical for both ratios. And in routine testing, the 1:8 cardiolipin-lecithin proved much more sensitive than did Kahn and Kolmer antigens. The comparative sensitivity results on 205

syphilitic serums were: cardiolipin 98 per cent, Kolmer 91.2 per cent, Kahn 83 per cent.

The 1:8 cardiolipin-lecithin showed a decided advantage over the 1:10 antigen in reducing the number of nonspecific reactions. In comparative tests with both ratios on 283 nonsyphilitic serums the 1:10 ratio showed 114 per cent more positive and 32 per cent more combined doubtful and positive reactions. More valid conclusions may be drawn from results of such parallel testing than from observations on separate groups of serums. It may be noted here, however, that in tests on 4453 presumably nonsyphilitic serums in the present series, the 1:8 cardiolipin-lecithin gave a specificity of 98.8 per cent, including 0.29 per cent false-positive reactions, while the results reported previously on 3652 serums tested with 1:10 cardiolipin-lecithin showed a specificity of only 97 per cent, with 1.16 per cent false-positive reactions.

The Kahn test showed a specificity of 99.3 per cent in the present series, but was considered less reliable because of the inadequate sensitivity in tests on low-titered serums. In the special cases of malaria, Kline's observations⁶ on the remarkably increased specificity of cardiolipin over Kahn antigen were confirmed (Klein and Leiby¹⁰).

SUMMARY

1. The Kline cardiolipin slide test affords a simple, reliable procedure for the serodiagnosis of syphilis.
2. The cardiolipin-lecithin ratio optimal for routine diagnostic testing was investigated.
3. The recommended cardiolipin-lecithin ratio showed an improved and practicable level of specificity, only slightly less than with the standard Kahn test.
4. The recommended cardiolipin-lecithin ratio gave significantly higher sensitivity results than did either the Kahn or Kolmer test.

Acknowledgment. The authors are grateful to Miss Mary Horne for her technical assistance.

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THE JUXTAGLOMERULAR APPARATUS OF THE HYPERTENSIVE KIDNEY*

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The juxtaglomerular apparatus has been described as a cuff of modified smooth muscle cells of the media of the afferent arteriole which are present for a variable distance along this vessel before it enters the glomerulus. With Masson's trichrome stain, these modified smooth muscle cells appear as large, pale-staining cells with large, prominent ovoid nuclei whose chromatin network is arranged in a somewhat radial pattern. They are afibrillar and their appearance has given rise to the term epithelioid cells. They are imbedded in a delicate fibrillar network, but, unlike similar-appearing cells in the carotid and aortic bodies, their innervation is no different from that of unchanged smooth muscle cells. While not prominent, they are occasionally seen in the wall of the afferent arteriole of the normal human kidney. In contrast, the efferent arteriole has only circular smooth muscle in its wall. Under certain hypertensive conditions these cells undergo hypertrophy and hyperplasia and are found to extend much farther along the afferent arteriole than its juxtaglomerular segment and even to be associated with the interlobular artery and the efferent arteriole. Under the same conditions, these cells take on acidophilic granules. These changes are most evident in the juxtaglomerular apparatuses of the outer cortical glomeruli.

Smith²² has summarized the early literature concerning the juxtaglomerular apparatus. The epithelioid cells have been described in the kidneys of the mouse, cat, dog, rabbit, and of man of all ages, and even of human fetuses. The initial concept of this structure was that its cells were derived from smooth muscle cells and that they functioned as a device for regulation of blood flow through the glomerulus. More recently, Goormaghtigh^{11, 16} has postulated that these cells exhibit endocrine activity and suggested that they are the site of renin production. In acute and subacute experiments with dogs and rabbits made hypertensive by the Goldblatt or Drury technic or by giving large doses of calciferol, he found transformation of smooth muscle cells into afibrillar epithelioid cells accompanied by mitotic activity, hypertrophy and hyperplasia, acidophilic and basophilic granulation, and vacuolization of these cells. He noted similar changes in human kidneys in cases of fulminating scarlet fever,⁸ eclampsia,^{9, 10} acute and subacute glomerulonephritis,¹³ early in the course of primary hypertension,¹³ and in the crush syndrome.^{14, 15} Kaufman^{15, 19} found hypertrophy and hyperplasia without granulation of the epithelioid cells in the kidneys of patients who showed clinical evidence of hypertension with progressive arteriolar nephrosclerosis, malignant nephrosclerosis, and chronic pyelonephritis with secondary vascular changes. Graef¹⁷ described similar findings in a kidney removed from a patient in her fifth month of pregnancy who subsequently

* Received for publication, July 23, 1948.

developed eclampsia. McManus²⁰ noted changes comparable to those described by Goormaghtigh in some 30 cases of glomerulonephritis.

MATERIALS AND METHODS

In this study the juxtaglomerular apparatuses of the kidneys of 100 patients obtained at autopsy were examined. These kidneys were selected because of their normal or hypertensive features. They were grouped according to their proven pathologic diagnosis as follows: (1) normal, (2) acute, subacute, and chronic glomerulonephritis, (3) eclampsia, (4) primary hypertension without uremia, and (5) primary hypertension with acute uremia.

Tissues were fixed in formalin, Helly's solution and Zenker's solution. The first two fixations were found to be preferable, although the fixative employed did not appear to be as important as the freshness of the tissue. A variety of stains was investigated since the granules of the epithelioid cells do not stain with hematoxylin and eosin and only faintly with phosphotungstic acid-hematoxylin and periodic acid. Masson's trichrome was satisfactory, although light green was substituted for aniline blue to differentiate connective tissue without obscuring cellular outline. The epithelioid cells with or without acidophilic granules in their afibrillar cytoplasm were well differentiated from the fibrillar smooth muscle cells, endothelial cells and fibrocytes. Azocarmine was found to be superior to Masson's trichrome in that the granules were stained intensely a brilliant red, but the differential features of afibrillar cytoplasm and chromatin pattern in cases of absent granulation were not so evident.

Sections to be examined were cut at five microns and several sections were mounted serially and studied with the oil immersion lens. Acceptable longitudinal cuts showed the afferent arteriole entering the glomerular tuft, and cross-sections were oriented as to the proximity to the vascular pole, relation to the macula densa, and location on the opposite side of Bowman's capsule from the origin of the proximal convoluted tubules.

RESULTS

1. Normal

A control group of 8 normal kidneys was examined. The ages of the patients varied from 9 to 62 years. The cause of death in six cases was trauma, in one case drowning, and in one rupture of a syphilitic aneurysm. Death was almost immediate in every instance. The blood pressure, the weight of the heart and

FIG. 1. Normal. Masson's trichrome stain. Afferent arteriole entering glomerulus. Note smooth muscle cells with small dark nuclei in the arteriolar wall adjacent to the glomerulus. No epithelioid cells are evident.

FIG. 2. Chronic hypertension without uremia. Masson's trichrome stain. Minimal evidence of epithelioid cells is seen in the wall of the afferent arteriole, but no granulation.

FIG. 3. Hypertension with acute uremia. Masson's trichrome stain. Note the marked hypertrophy and hyperplasia of epithelioid cells, eccentric proliferation forming tumor-like nodule, and marked granulation.

FIG. 4. Malignant hypertension. Azocarmine stain. Note the marked granulation and hypertrophy and hyperplasia of the epithelioid cells in the juxtaglomerular segment of the afferent arteriole.



FIGS. 1-4

the gross and microscopic appearances of the kidneys were normal. No granules and no epithelioid cell hypertrophy or hyperplasia were noted in this group. At the juncture of the afferent arteriole and the glomerulus, one or several epithelioid cells were occasionally seen but these were not prominent or evident in all the vessels examined.

2. Glomerulonephritis

Four cases of acute glomerulonephritis were examined. The duration of symptoms ranged from five to ten days and hypertension was present in two of three recorded instances. Gross and microscopic appearances of the kidneys were consistent with the diagnosis of acute glomerulonephritis. In three kidneys, the epithelioid cells showed moderate, and in one, minimal but definite, hypertrophy and hyperplasia. One kidney showed acidophilic granules and another a prominent vacuolated cell, a change which Goormaghtigh considers to be a phase in the granulation cycle.

Three cases of subacute glomerulonephritis were studied in which the duration of symptoms ranged from two to eight months. All had hypertension, with moderate or high levels of blood urea nitrogen, some increase in cardiac weight, and compatible findings in the kidney. In two cases, there was minimal and in one, no epithelioid hypertrophy and hyperplasia. In no instance was there granulation of the epithelioid cells.

Five kidneys were examined from patients with chronic glomerulonephritis in four of whom the duration of the disease ranged from 13 months to 5 years and one was recorded as 31 years. Hypertension, elevated blood urea nitrogen, cardiac hypertrophy and renal atrophy were constant findings. In two of the five there was only minimal epithelioid cell hypertrophy and hyperplasia and none of the kidneys showed granulation.

3. Eclampsia

The kidneys from five patients with eclampsia were examined, in whom the duration of symptoms ranged from two hours to two months before death. All five patients exhibited convulsions and had four-plus albuminuria. Four of the five had hypertension, and in three, the recorded values of the blood urea nitrogen were normal or slightly elevated. Two of the patients showed marked epithelioid cell hypertrophy, one, moderate, and two, minimal change. In three kidneys granulation was evident. The kidney from the patient without hypertension showed slight epithelioid cell hypertrophy and hyperplasia and no granulation.

4. Chronic Hypertension without Uremia

Thirty-five kidneys were studied from patients who clinically and pathologically were diagnosed as having primary hypertension without uremia or benign hypertension. Eleven patients died from cerebral vascular lesions, 15 from myocardial infarction or coronary insufficiency, 6 from myocardial failure, 2 from pulmonary embolism, and one from mesenteric thrombosis. Their ages varied from 44 to 80 years.

All patients in this group exhibited hypertension or cardiac hypertrophy or both. None presented the clinical features of uremia, and the available urea nitrogen levels were normal or only slightly elevated. The combined weight of both kidneys varied from 240 to 550 grams. Microscopically the walls of the small arteries and arterioles either showed no histologic alterations or splitting of the internal elastic membrane and hyaline degeneration without collagenous intimal thickening.

The juxtaglomerular cells showed no hypertrophy or hyperplasia in the kidneys of 25 patients, slight or minimal change in 9 and a moderate alteration in one. The kidneys of 33 patients exhibited no granulation, and in two patients, both of whom had myocardial infarction, the kidneys showed acidophilic granules in the hyperplastic epithelioid cells.

5. Hypertension with Uremia

The kidneys of 40 patients with primary hypertension who died in uremia were examined. Patients with chronic hypertension terminating in acute uremia and others with rapidly progressive hypertension with uremia, *i.e.*, malignant hypertension, were included in this group. The age of the patients varied from 26 to 73 years. The period of labile blood pressure varied greatly but the duration of symptoms was relatively short. Blood pressures were above normal in all cases and were higher than in those cases with primary hypertension without uremia. Elevated levels of blood urea nitrogen were recorded in 35 cases. Cardiac hypertrophy was present in all patients, except one with mitral stenosis. Retinitis was present in 25 of 26 recorded instances. Microscopic examination of the kidney showed some or all of the diagnostic features of collagenous intimal thickening of the small arteries and arterioles, thrombocytosis, and focal glomerulitis.

Epithelioid cell hypertrophy and hyperplasia were evident in all 40 cases and were most prominent in this group of patients. In 8 patients, the changes were considered to be minimal, in 20 moderate and in 12 marked. In 34 cases the epithelioid cells showed granulation, in 4 the presence of granulation was questionable and in 2 there were no granules. The two patients without granulation of the epithelioid cells had symptoms for only three weeks before death, and their urea nitrogen levels were comparatively low for this group. In general the degree of hypertrophy and hyperplasia of the epithelioid cells paralleled the presence and intensity of granulation, and both changes were particularly prominent in the juxtaglomerular apparatuses associated with the outer cortical glomeruli of the kidneys of those patients in the younger age group with a more rapid progression of symptoms.

COMMENT

Granulation and hypertrophy and hyperplasia of the juxtaglomerular epithelioid cells were noted in acute glomerulonephritis, eclampsia, and primary hypertension with acute uremia. These changes were minimal or absent in the more chronic conditions of subacute and chronic glomerulonephritis, and primary

hypertension without uremia. Most noteworthy is the marked contrast in the findings observed in the two groups of primary hypertension. Patients with benign hypertension showed minimal or no alteration of the juxtaglomerular cells, whereas those with malignant hypertension showed the most marked and consistent changes. The juxtaglomerular apparatuses which showed the most granulation also showed the most hypertrophy and hyperplasia of their cells, and both changes were most prominent in association with the outer cortical glomeruli. These observations are, in general, in agreement with those described in the literature, *viz.*, granulation, hypertrophy and hyperplasia of the juxtaglomerular cells occurring in association with acute hypertensive states.

Goormaghtigh¹² has suggested repeatedly that the epithelioid cells exhibit a secretory cycle and that their granules may represent the formation of a vaso-pressor substance, probably renin. Correlation exists between anatomic alterations of the juxtaglomerular apparatus and positive renin assays of the blood in cases of acute glomerulonephritis, eclampsia, and malignant hypertension, and negative findings in cases of subacute and chronic glomerulonephritis, and benign hypertension. Evidence presented by Weeks, Steiner, Mansfield, and Victor²⁴ and by Friedman and Kaplan⁴⁻⁷ indicates that the proximal convoluted tubule and not the juxtaglomerular apparatus is the site of renin formation. Braun-Menéndez and co-workers¹ have pointed out that Goormaghtigh's hypothesis does not explain the immediate liberation of renin which follows experimental renal ischemia.

What the granules of the juxtaglomerular cells are, and what their significance is, can only be surmised. Their presence is not related to the level of the blood urea nitrogen. Increasing evidence indicates that renin is only one of a number of pressor agents with which the kidney is concerned. Such substances or their anti-substances may account for the granulation. Finally the granules may represent degeneration and extrusion of fibrillar material as the smooth muscle cell becomes modified and changes over to the afibrillar epithelioid cell.

The juxtaglomerular apparatus was originally considered to function, in a manner similar to the glomus structure of the skin, as a shunt which regulated the blood flow through the glomerulus. If this be the case, then changes in cellular pattern, such as hypertrophy and hyperplasia, should be accompanied by alterations of the blood flow through the kidney.

Dock³ has shown that perfusion rates through kidneys of hypertensive patients dying of stroke, coronary disease, and heart failure are equal to those of normotensive persons of the same age group, whereas the perfusion rates are markedly decreased in patients with uremia. Chasis and Goldring² in their study of patients with primary hypertension by the use of clearance methods found decreased renal blood flow late in the course of the disease.

Wellen and co-workers²⁵ failed to find a reduction in renal blood flow in toxemia of pregnancy. Braun-Menéndez *et al.*¹ also found normal blood flow but a diminution of glomerular filtrate in eclampsia. This observation is compatible with a short-circuit of the blood through an alternate circulation. The fact that cortical necrosis of the kidney in eclampsia is noted occasionally lends weight to this explanation.

Trueta and his associates²³ point out that their studies of renal circulation have shown that large numbers of glomeruli may, in fact, be excluded from the circulating blood by way of a deep intra-renal by-pass through the juxtamedullary glomeruli and the vasa recta. The glomeruli of the alternate circulation may be less efficient filters and more efficient shunts as evidenced by the comparatively large caliber of their efferent arterioles and the frequent degeneration of their capillary loops to give virtual arterioles.

Smith²² has emphasized the large degree of independence of the renal circulation and considers the renal blood flow to be regulated entirely by the autonomous activity of the renal arterioles. Both Goormaghtigh¹⁰ and Okkels²¹ have attributed independent action to the juxtaglomerular apparatus. Thus a mechanism exists within the kidney for regulating the flow of blood between cortex and medulla. Ischemic atrophy of the kidney in the late stages of primary hypertension predominates in the outer cortex where the granular epithelioid cells are evident and where their hypertrophy and hyperplasia are most marked.

From these observations concerning blood flow in the hypertensive kidney some correlation exists between alterations of the blood flow and the changes in the histology of the juxtaglomerular apparatus. Therefore, this structure may serve as a mechanism to regulate blood flow through the cortical and juxtamedullary circulatory systems, and hypertrophy and hyperplasia of its cells reflect an increased importance and work in the hypertensive conditions under which they are found.

SUMMARY

1. The juxtaglomerular apparatuses of normal and hypertensive kidneys obtained at autopsy from 100 patients were examined with Masson's trichrome and azocarmine stains.

2. In general, the observations concerning the juxtaglomerular apparatus as reported by various authors were confirmed. Granulation, hypertrophy and hyperplasia of the epithelioid cells were observed in patients with acute and progressive hypertension terminating in acute uremia. These changes predominated in the outer cortex of the kidney. A significant difference exists in cellular response between cases of benign and of malignant hypertension.

3. The hypothesis of Goormaghtigh that the epithelioid cells of the juxtaglomerular apparatus are the site of renin formation cannot be supported in view of other physiologic and anatomic findings. The nature and significance of the acidophilic granules remains unknown.

4. Analogy of the juxtaglomerular apparatus to the glomus structure of the skin has been made, and an alternate intrarenal circulation has been described. The suggestion is made that juxtaglomerular apparatus acts as a mechanism for regulating blood flow through the cortical and juxtamedullary circulations, and that this structure undergoes changes which reflect increased importance and work in acutely hypertensive states.

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FATAL HEMATEMESIS FROM RUPTURE OF AORTIC ANEURYSM INTO CARCINOMA OF ESOPHAGUS*

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The following case of fatal hemorrhage due to intercommunication of an aneurysm of the descending aorta and an esophageal carcinoma is presented because of its extreme rarity.

REPORT OF CASE

A 50 year old white male, a brewery worker, was admitted to this hospital in October 1946. For one and one-half years prior to entry he had noted postprandial burning pain located just above the xiphoid. He recently had lost 24 pounds in weight and developed fatigability and anorexia. Three months prior to entry, he began to experience dysphagia with regurgitation of solid food, and complained of dull persistent pain in the upper part of the back and mild dyspnea on effort. He gave a history of a penile chancre thirty years ago for which he received only local treatment.

Physical examination revealed evidence of recent weight loss. A soft subcutaneous tumor, 2.0 cm. in diameter, was noted in the left occipitoparietal region of the skull. The pupils were miotic and equal and reacted to accommodation but not to light. No cardiac abnormalities were detected. The blood pressure measured 135/85 mm. of mercury in each arm. A small penile sear was detected near the frenulum. The right Achilles tendon reflex was depressed. The gait was normal and Romberg's sign was absent.

The hemoglobin was 16.6 Gm. per 100 ml. blood, the erythrocyte count 4,720,000 per cu. mm., the leukocyte count 12,250 and the differential count normal. The blood Wassermann and Kahn tests were negative on two occasions. Urinalyses were normal. The cerebrospinal fluid gave a negative globulin test, contained 13 white blood cells and two red blood cells per cu. mm.; the colloidal gold test and the Wassermann test were negative.

Röntgen examination of the chest showed diffuse dilatation of the thoracic aorta. A calcified aneurysm of the proximal descending aorta measured about 6.0 cm. in diameter and displaced the trachea and left main bronchus to the right. The fusiform dilatation of the descending portion had produced pressure atrophy of the ninth thoracic vertebra. Barium study of the esophagus showed irregular narrowing and constriction of the lower third, consistent either with intrinsic malignancy or extrinsic pressure from the known aneurysm of the thoracic aorta. An irregularly round, circumscribed bony defect of the left lateral part of the occipital bone was seen by x-ray. It lay beneath the soft tissue nodule of the scalp previously described and measured 1.5 cm. in diameter. Esophagoscopy examination disclosed an infiltrating tumor of the middle third of the esophagus. Biopsy of tissue from this region demonstrated no evidence of malignancy, but biopsy of tissue aspirated from the occipital mass revealed secondary squamous cell carcinoma. Two months after entry, the patient died of exsanguination following a sudden massive hematemesis.

Postmortem Findings

At postmortem examination, performed four and one-half hours after death, the skin showed marked pallor. The significant gross findings were confined to the esophagus and

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aorta. The middle third of the esophagus was the site of an annular carcinoma. The lower margin of the tumor was 10 cm. from the cardia and the tumor varied from 1 to 4 cm. in width. The wall of the involved portion of the esophagus averaged 1.2 cm. in thickness and the adhesions between it and the underlying aortic aneurysm were dense and firm. The luminal surface of the neoplasm was for the most part smooth, firm and nonulcerated except for an area at the lower posterolateral margin of the tumor. Here an ulcerated zone filled with friable necrotic material extended toward the thoracic aorta. The aorta showed a combination of extreme atherosclerosis and syphilis. The atherosclerotic plaques were diffusely scattered throughout all portions of the aorta; they were frequently calcified and a few in the abdominal portion were ulcerated. The intima of the arch and thoracic portions of the aorta, between the atherosclerotic plaques, showed the characteristic longitudinal wrinkling of syphilitic aortitis, and these portions of the aorta were dilated and tortuous. The thoracic aorta, just beyond the arch, was the seat of a broad saccular aneurysm which measured 6 cm. in length, 4 cm. at its widest point and 2 cm. at its deepest point. The rim of the aneurysm was smooth and rounded; the floor was composed of a large calcified plaque with raised edges. The thickened wall of the aneurysm was adherent to the tumorous portion of the esophagus. Sections through this commonly-shared connective tissue showed gross evidence of tumor with small areas of necrosis. Just beneath the lower rim of the calcified floor of the aneurysm, the wall of the aneurysmal sac was disrupted. This break in the wall of the sac was continuous with the deeply ulcerated portion of the esophageal cancer. The relation of these lesions to the immediate cause of death was demonstrated by the presence in the stomach of two large blood clots, each with a volume of approximately 500 cc., and by a large amount of fluid and clotted blood in the small and large bowel. In addition, there was a metastatic tumor nodule in the left occipital region which had eroded through the skull and was adherent to the underlying dura and a small tumor nodule in the cortex of the right kidney.

Microscopically, the esophageal tumor, the nodule in the skull, and the nodule in the right kidney all showed squamous cell carcinoma, grade 2. Sections through the region of the esophageal aortic fistula showed complete necrosis of the esophageal mucosa and submucosa. The zone of tumorous necrosis extended down through the muscular layers and into the peri-esophageal connective tissue, the latter being indistinguishable from the broadened adventitia of the aortic aneurysm. This loose to dense sheet of aortic adventitia and peri-esophageal neoplastic tissue showed numerous small zones of necrosis. Islands of squamous carcinoma extended to but not beyond the external elastica, and the blood vessels in this area showed collars of lymphocytes and plasma cells. The media of the aorta was thinned and composed of elongated connective tissue fibers with a sprinkling of lymphocytes and monocytes; smooth muscle elements were lacking in this layer. The intimal remnants were composed of small zones of a loose amorphous hyaline material. At the site of the tear in the aneurysmal sac all remnants of intima and media were lacking. Sections from other portions of the aorta showed evidence of syphilis and severe atherosclerosis. The only other microscopic findings worthy of note were the presence of tumor in the lungs and liver and moderate cerebral edema. The postmortem diagnoses were: (1) Squamous cell carcinoma of esophagus, grade 2, with metastasis to skull, lungs, liver and right kidney, and (2) aneurysm of thoracic aorta, luetic, with rupture and esophageal-aortic fistula.

DISCUSSION

Saccular aneurysms of the thoracic aorta not infrequently perforate the esophagus.^{2, 6} Of 1,197 ruptures of thoracic aneurysms into various organs,² the esophagus was the third most common site involved (9.4 per cent). Of 95 such ruptures in Kampmeier's series,⁶ perforations of the esophagus were second in order of frequency (22.1 per cent). This complication occurs with greatest relative frequency in aneurysms of the transverse and descending aorta.^{2, 6}

Perforations of an aneurysm into the esophagus may occur slowly, resulting in oozing of blood over a long period of time. In this situation an erroneous diagnosis of carcinoma of the esophagus or stomach is not infrequently entertained. In the group of 4,465 cases of aortic aneurysm collected by Boyd² and Kampmeier⁶ no instance of perforation into an esophageal carcinoma was recorded.

Carcinoma of the esophagus frequently erodes into contiguous structures, especially the trachea, bronchus, mediastinum or lung.¹ These tumors rarely rupture into the aorta, only 60 such cases being recorded up to 1946.⁸ This group includes only two cases of perforation of an aortic aneurysm by an esophageal cancer.³

Dalous and Fabre⁴ record two cases of coexistent cancer of the esophagus and aneurysm of the aorta. They emphasized the great rarity of this finding and they could find no similar cases. In the second patient the neoplastic cells had invaded all the tunics of the aorta, resulting in a fistula between the aneurysm and the esophagus with subsequent fatal hemorrhage. This brings to three the total known recorded cases of intercommunication of an aneurysm of the aorta and a cancer of the esophagus. Because of the presence of syphilis in both of the cases reported by Dalous and Fabre, these authors felt that it played an essential role in the production of the esophageal cancer. No statistical relationship between these two diseases is shown in large series of cases of esophageal neoplasm.^{5, 7, 10}

Schattenberg and Ziskind⁹ felt that perforation of the aorta by carcinoma of the esophagus usually occurs either as a result of disintegration of the tumor before an advancing ulcerative process, or in some cases directly from suppuration. Postoloff and Cannon⁸ presented two cases in which perforation was not due to invasion of the entire wall of the aorta by tumor, only the adventitia being involved by neoplastic tissue. Perforation of the aorta resulted from interference of the blood supply secondary to thrombosis of the vasa vasorum. Although bacteria were seen in microscopic sections, they were probably saprophytes, since no fibrin or inflammatory cellular reaction was present at the site of perforation.

The case here presented is one of perforation of a syphilitic aortic aneurysm into a deeply ulcerated carcinoma of the esophagus. Microscopic evidence showed that the aneurysm, at a point where the intima and media were completely lacking, had ruptured into a zone of tumor necrosis. At no point did the neoplasm penetrate beyond the aortic adventitia, nor was there evidence of thrombosis of the vasa vasorum.

SUMMARY

A case of fatal hemorrhage due to intercommunication of an aneurysm of the descending aorta and an esophageal carcinoma is presented. Review of the literature disclosed only three similar cases.

In this instance, there was perforation through the weakened portion of the aneurysmal wall into a zone of necrosis of the esophageal neoplasm.

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CYANIDE POISONING

REPORT OF A CASE WITH RECOVERY*

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The annual number of reported deaths from cyanide poisoning in the United States since 1909 has varied from 79 to 245. It is commonly believed that cyanide poisoning is invariably fatal. Twenty-two cases of recovery after ingestion of sodium or potassium cyanide have been reported in the American, English and French literature.^{7-9, 20, 27, 29, 33, 37} Additional cases^{20, 23, 25, 36, 37} of recovery from poisoning by cyanide from routes other than ingestion, including a number in which silver polish was presumed to have been the toxic agent, have also been reported. Generally, it is difficult to ascertain the exact quantity of cyanide that has entered the body. The patient's own testimony is notoriously unreliable, and usually there is no adequate determination of the content of cyanide in the patient's blood or urine. As a result, it is often impossible to determine the severity of the intoxication.

The purpose of this paper is to report our findings in a patient who recovered following the ingestion of an unusually large amount of potassium cyanide, which was estimated to have been between 3 and 5 Gm.

REPORT OF CASE

C. S., a 60 year old white male chemist, was admitted to the psychiatric division of Bellevue Hospital one hour after ingestion of potassium cyanide in a suicidal attempt. The patient vomited once, one-half hour after taking the poison.

On admission he was comatose, did not react to painful stimuli and showed generalized muscular rigidity. His temperature was 97 F., blood pressure 120/80, pulse rate 140 and respiratory rate 40 per minute. The respirations were stertorous, deep and rapid, with an expiratory grunt. The skin was pink, cold and clammy, and the pupils were dilated. The pulse was thready, and the heart sounds were inaudible.

No history was available at the time, but acute poisoning was suspected. Gastric lavage was performed immediately, using four liters of 10 per cent solution of sodium bicarbonate. An infusion of 1000 ml. of 5 per cent glucose in normal saline was started, and an injection of 40,000 units of penicillin was administered intramuscularly every three hours.

One-half hour later the patient regained consciousness. The clinical course was marked by gradual improvement. Eight hours after admission he was alert, well oriented and cooperative. His only complaints were weakness and nausea. The pink color of the skin had begun to fade, the respirations had decreased to 26 per minute and were less labored, but the pulse rate remained elevated and the blood pressure was 168/104. At this time examination revealed the heart to be enlarged with the point of maximal impulse in the fifth interspace just outside the midclavicular line. The rhythm was regular, and a harsh apical systolic murmur was transmitted to the left sternal border. The patient improved rapidly and was discharged on the fifth hospital day. Six months later he was in good health and had resumed his professional work.

* Received for publication, April 2, 1948.

LABORATORY DATA

Shortly after admission, a qualitative test on the gastric washings showed cyanide to be present in large amounts. The material, in a bottle on the patient's person, was found to be potassium cyanide. Blood cyanide determinations revealed 20 mg. of hydrogen cyanide per 100 ml. of blood in the specimen drawn two hours after admission to the hospital, making an estimated total of 1.2 Gm. of hydrogen cyanide in the total blood volume. Thirteen hours after admission the tests revealed the presence of 0.25 mg. of hydrogen cyanide per 100 ml. of blood, making an estimated total of 13.0 mg. of hydrogen cyanide in the blood stream at that time (Table 1). A qualitative test for methemoglobin was negative. Two hours after admission, the carbon dioxide combining power of the plasma was 25 volumes per 100 ml. and rose to 44 volumes on the third

TABLE 1
CYANIDE DETERMINATIONS IN BLOOD AND BODY

APPROXIMATE TIME AFTER INGESTION	BLOOD LEVEL OF HYDROGEN CYANIDE*	ESTIMATED TOTAL HYDROGEN CYANIDE IN BLOOD	ESTIMATED TOTAL HYDROGEN CYANIDE IN BODY	ESTIMATED HYDROGEN CYANIDE/KG. BODY WEIGHT
hr.	mg./100 ml.	mg.	mg.	mg.
2	20.0	1200.0	2400	30.0
13	0.25	13.0	26	0.325
24	0.0	0.0		
48	0.0	0.0		
72	0.0	0.0		
96	0.0	0.0		

* Determined by the method of Gettler and Goldbaum.¹¹ The blood is acidified and aerated. The gases are passed through a small disc of filter paper impregnated with a mixture of ferrous sulphate and sodium hydroxide. The paper is then treated with dilute hydrochloric acid, dissolving out the iron hydroxides and forming a blue color, the intensity of which varies proportionately with the amount of cyanide present.

hospital day. The basal metabolic rate eight hours after admission was plus 70 per cent and plus 17 per cent on the fifth hospital day. An electrocardiogram on the second hospital day showed normal sinus rhythm with left axis deviation. An x-ray film of the chest showed enlargement of the heart in all diameters with straightening of the left cardiac border; the lungs were clear. On the fourth day of hospitalization, the red cell count was 6,210,000 per cu. mm., the hemoglobin value 17 Gm. per 100 ml. of blood, the hematocrit 50 and the sedimentation rate 16 mm. in one hour (Wintrobe method). The blood Wassermann test was negative. Urinalysis on the day of admission revealed a pH of 4.5 and albumen, one plus; on the third hospital day, the pH of the urine was 7.0, and there was no albuminuria.

The blood sodium, chlorides, total proteins, albumin-globulin ratio, inorganic phosphorus and total and esterified cholesterol determinations on the fourth hospital day, were all within normal limits. Blood thiocyanate determinations on the first, second, fourth and fifth hospital days, were negative. Urinary

thiocyanate excretion was determined on the first, second and third days with the results shown in Table 2. Free cyanide in the second twenty-four hour specimen of urine was found to be 0.116 mg. in 1160 ml. of urine.

COMMENT

Two hours after ingestion of an estimated 3 to 5 Gm. of potassium cyanide there were at least 1.2 Gm. of hydrogen cyanide in the patient's vascular system.† Assuming there was twice this amount in the entire body,^{8, 10} and since the patient weighed approximately 80 Kg., the concentration of hydrogen cyanide would be 30 mg. per Kg. This is about thirty times the estimated lethal dose.^{2, 10} A second blood determination eleven hours later confirmed the presence of cyanide with an estimated concentration of 0.325 mg. per kilogram.

The test for thiocyanate in the blood was negative. Urine determinations revealed the patient had excreted 237 mg. thiocyanate (equivalent to approxi

TABLE 2
ESTIMATION OF URINARY THIOCYANATE EXCRETION*

24 HOUR COLLECTION	URINE VOLUME	THIOCYANATE	TOTAL THIOCYANATE
	ml.	mg./100 ml.	mg.
1st	1540	2.01	30.9
2nd	1160	10.2	118.32
3rd	1570	5.6	87.92
Total over 72-hour period.....			237.14

* Thiocyanate in the urine was determined as follows: Proteins are precipitated by trichloroacetic acid and the thiocyanate in the filtrate determined colorimetrically with ferric nitrate. The tests were performed within one hour after receiving the urine.

mately 110 mg. of hydrogen cyanide) in a seventy-two hour period. The normal average amount of thiocyanate in urine varies between 0.85 and 14 mg. per twenty-four hours.²²

The patient was questioned repeatedly and denied taking any antidote or having any knowledge of exposure to any substance that might have had an antidotal effect. He used sodium thiosulphate in his daily work, immersing his hands in it frequently.

With the above data it has been impossible for us to arrive at an adequate explanation for his recovery. It has been suggested that cyanide combines with glucose.³ He received 50 Gm. of glucose in the first infusion. We can assume that he had approximately 9 Gm. available in his blood prior to the infusion (on the basis of a blood sugar of 150 mg. per 100 ml. and a blood volume of 6 liters). This makes a total of 59 Gm. available for conversion of an estimated 1.2 Gm. of cyanide to the nontoxic cyanohydrins. Fifteen Gm. of glucose is sufficient for conversion of 5 Gm. of cyanide to cyanohydrins in *in vitro* experiments.

† The blood volume was assumed to be 6 liters for this calculation.

CLINICAL PICTURE OF ACUTE CYANIDE INTOXICATION

The clinical picture of acute cyanide poisoning has been described by many authors, with disagreement as to the characteristic signs and symptoms. Death may occur almost immediately after taking a fatal dose, or it may be delayed for more than three hours.¹⁰ Common symptoms described are unconsciousness, deep stertorous respirations, nausea and a state of agitation when the patient is conscious. The reported signs are dilated pupils, flushed skin with cyanosis of lips and nail beds, tachycardia and hypotension leading to shock and convulsions. Acetone is frequently found in the urine. Oxygen consumption is increased.³² Free cyanide may be found in blood and urine recovered from the living patient as well as in all tissues at autopsy.¹⁰ Blood and urinary thiocyanate levels may be elevated.

Haines¹² described the execution of a prisoner (Robert H. White) in a hydrogen cyanide gas chamber in 1930. At the beginning of the execution the subject appeared normal except for a pulse rate of 108. One-half minute following the beginning of exposure to the gas he took a deep inspiration which was followed by a spasmodic cough. Respirations then became stertorous and irregular and he shortly lapsed into unconsciousness. This was followed by a complete cessation of heart action for fifteen seconds, after which the heart began to beat again irregularly. Nine and one-half minutes following the beginning of exposure to the gas, the heart action stopped altogether, and respirations ceased shortly thereafter.

Treatment

Treatment, on the whole, has been far from satisfactory, because cyanide poisoning is usually rapidly fatal. Methylene blue was one of the first drugs used for cyanide intoxication. At first, methylene blue was believed to act as a respiratory catalyst,¹⁰ but more recently it has been claimed that its specific antidotal effect was in producing methemoglobinemia.^{13-16, 24, 26, 34, 35} The methemoglobin then combines with the cyanide ion to form the relatively non-toxic cyanmethemoglobin. The latter is believed to be slowly converted by sulfhydryl groups to thiocyanate and eliminated as such. Glucose is believed to have a definite antidotal effect. Animal experimentation has shown that a nontoxic cyanohydrin is slowly formed by dextrose and cyanide ion.^{4, 5, 21, 28} Chen, Rose and Clowes² and, subsequently, others^{1, 3, 17-19, 30, 31, 34} in a series of experiments on dogs, studied the relative merits of methylene blue and amyl nitrite, sodium nitrite and sodium thiosulphate. They found that combined therapy with amyl nitrite, sodium nitrite and sodium thiosulphate was 5 to 6 times as effective as methylene blue treatment alone in protecting dogs from otherwise fatal doses of cyanide.

On the basis of the above considerations, the currently favored treatment² consists of the immediate administration of amyl nitrite pearls, followed by intravenous injection of from 0.3 to 0.6 Gm. of sodium nitrite and, finally, of intravenous injection of 25 to 50 Gm. of sodium thiosulphate. Treatment may be repeated, if necessary, using one-half of these quantities.

Excess methemoglobin formation from nitrite administration may be ascertained roughly by an increasing cyanosis despite clinical signs of improvement and may be combatted by repeated blood transfusions and the administration of oxygen.

SUMMARY

A case is reported of recovery from cyanide poisoning. A quantity of cyanide was absorbed far in excess of the commonly accepted minimum lethal dose. Quantitative blood determinations of hydrogen cyanide were made.

No specific therapy was given, and recovery was prompt and uneventful.

The symptomatology and treatment of cyanide poisoning are outlined.

Acknowledgment. The authors wish to thank Dr. A. Walter Freireich for his generous advice and assistance in the preparation of the manuscript.

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DEATH FOLLOWING INGESTION OF FERROUS SULFATE*

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Ferrous sulfate is regarded as a relatively nontoxic substance. Reports of poisoning with iron salts are scarce. Helpern⁷ in 1937 reported 898 deaths from accidental, homicidal and suicidal poisonings, but no case of poisoning from iron salt was included. Several cases of ferrous sulfate poisoning have been reported^{1, 2, 4-6, 8} in both foreign and American journals during the period from 1850 to 1890. Peterson, Haines and Webster⁹ refer to a death after ingestion of 45 cc. of tincture of iron, equivalent to 6 Gm. of salt, but make no reference to the pathologic findings. They also mention four cases of homicidal poisoning in Martinique with ferric chloride. Necropsy of one case showed a greenish black fur-like "mud" covering the tongue, esophagus and stomach; swelling, congestion and ecchymotic points in the liver and kidneys, and marked hyperemia of the brain and membranes.

The pharmacologic actions of iron have been studied extensively, but studies on the toxicologic effects of iron salts are meager. Edmunds and Gunn in Cushny's Pharmacology³ describe the effects of oral ingestion of large quantities of iron salts as consisting of "pain and uneasiness in the stomach, nausea, vomiting and often purging, with all the ordinary symptoms of gastrointestinal irritation. General weakness and collapse may be induced, but are manifestly secondary to the gastrointestinal irritation; and no symptoms which may be attributed to the absorption of iron have been observed in either man or animals." We have had an opportunity to study a case in which ferrous sulfate was ingested, and we were able to confirm the above findings that death resulted from secondary shock following the ingestion of a strong corrosive agent. Iron was important in this case only as the vehicle of an anion that constituted a strong acid.

REPORT OF CASE

Clinical data. A white male, age 26, was admitted to the hospital in shock with a history of having accidentally ingested one-quarter ($\frac{1}{4}$) pound of ferrous sulfate U.S.P. in aqueous suspension. He was cyanotic, had vomited blood and was doubled up with abdominal pain. The skin presented cyanosis and purplish blotches. The scleral blood vessels were congested, and the pharynx was injected. There were blood stains about the mouth, nose and pharynx. Examination of the chest was negative. No arterial pulse was palpable, nor could a blood pressure reading be obtained. The heart was not enlarged to percussion, the sounds were heard faintly and the rate was about 130 per minute. The abdomen was not distended, but there was boardlike rigidity. No peristalsis was felt or heard. A dark brownish black liquid was oozing from the anus. The reflexes were normal. The red blood cell count was 7,860,000, hemoglobin 114 per cent, leukocyte count 55,750 with a differential count of neutrophils 74 per cent, lymphocytes 25 per cent and eosinophils 1 per

* Received for publication, April 12, 1948.

cent. The treatment consisted of gastric lavage, oxygen, transfusion with whole blood and artificial respiration. The patient expired after about three hours.

Autopsy findings. The pupils were slightly dilated. The gums were discolored dark brown, and the nail beds were cyanotic. The muscles were of normal color. The peritoneum was dusky red and smooth, and there was a small amount of sanguineous fluid in the pelvic cavity. There were small, dark red, soft lymph nodes along the greater curvature of the stomach. There was a small amount of sanguineous fluid in the left pleural cavity. The pericardium was dusky red in color. Examination of the *lungs* revealed hemorrhage in the left lower lobe. The other lobes were edematous. The bronchi were congested and contained a frothy fluid. The *heart* showed subendocardial hemorrhage throughout the left ventricle. The *spleen* was grossly normal. The *liver*, *pancreas* and *adrenals* displayed no gross abnormalities. The *esophagus* showed erosions of the mucous membrane and a grayish substance adherent to the lining of the distal end. The *stomach* was dilated and filled with dark, bloody, thick fluid. The gastric wall showed large areas of hemorrhage. The rugae were obliterated and the mucous membrane was discolored gray and red, extensively eroded and covered with adherent grayish black, metallic, granular substance. Similar erosions and grayish black content were noted in the *duodenum* and upper portion of the *jejunum*. The mucous membrane of the rest of the intestinal tract was congested and covered with grayish black granular material. The large intestine contained reddish black fluid material, and the mucous membrane was covered with plaques of grayish black material. The *genitourinary* tract was normal on gross examination. The *dura* and *dural sinuses* were normal. The cerebral vessels were normal. The *brain* and ventricles were not remarkable.

Microscopic examination of the *lungs* showed a filling of the alveoli and bronchioles of the left lower lobe with whole blood. There was no pneumonic reaction. The epithelium of the trachea was desquamated. Sections of the *stomach* showed necrotic mucosa covered with a granular mantle. There was congestion, hemorrhage and edema, and lymphocytic infiltration throughout the substantia propria mucosae. The submucosa was congested. The epithelium of the *jejunum* and *ileum* showed necrosis with deposition of coarsely granular material. The submucosa presented congestion and edema. The *liver* showed varying degrees of acute parenchymatous degeneration. The cytoplasm was finely granular. Many cells were without nuclei while other liver cells displayed large hyperchromatic nuclei. The *spleen* displayed congestion and hemorrhage of the red pulp. The interlobular fatty areolar tissue of the *pancreas* presented edema and varying degrees of hemorrhage; no fat necrosis was identified. Sections of the *adrenals* were normal. The *kidney* sections showed congestion of the glomerular capillaries. The cells lining the convoluted tubules showed finely granular degeneration and many cells had no nuclei. The lumina of the convoluted tubules were small and contained a finely granular, acellular detritus. In the glomerular zones of the medullary rays (deep cortex), there were a few areas of interstitial lymphocytic infiltration. Examination of the sections of the *forebrain* showed subarachnoid congestion, edema of the cerebral cortex and pyknosis of the pyramidal cells. No hemorrhage and no perivascular cellular infiltrations were noted. The *basal ganglia* showed venous and capillary congestion. Sections of *medulla* displayed subependymal edema. There were no changes in the hypoglossal, vagal areuate or inferior olivary nuclei, or in the *pituitary*.

Prussian blue reaction. (Ferrous sulfate is unstable and is oxidized to ferric sulfate.) The mantle attached to the surface of the gastric mucosa displayed a Prussian blue reaction. There was aspiration of gastric content in the lungs. The alveolar walls displayed a Prussian blue reaction involving the endothelium of the capillaries and the cytoplasm of included granulocytes. Sections of kidney, brain, thyroid, liver and adrenal all gave a negative Prussian blue test for ferric iron. The residue in drinking glass contained ferrous sulfate. The vomitus contained large amounts of ferrous ions, small amounts of ferric ions and large amounts of sulfate radical. The contents of the stomach and large intestine revealed large amounts of blood, ferrous and ferric ions and sulfate radical. Thus, the Prussian blue test was positive only in those tissues which were directly in contact with the ferrous sulfate.

SUMMARY

The oral ingestion of one-quarter pound of ferrous sulfate was followed by death within three hours. The symptoms were those of very severe gastrointestinal irritation, and death was attributed to shock. There was no clinical, pathologic, or toxicologic evidence of absorption of the ferrous sulfate.

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CLINICOPATHOLOGIC CONFERENCE*

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CLINICAL DATA

History. A 22 year old soldier was admitted to the hospital complaining of mental and physical sluggishness. His past medical history and family history were essentially negative. Six months prior to admission he first noted swelling and reddish discoloration of the face, weakness of the legs, fatigue and exhaustion. About that time there also developed nocturia, loss of sexual desire, sluggish cerebration, and loss of ambition. The abdomen became puffy and he suffered from dull frontal headaches. He was aware of these changes which worried him. He became stooped and felt like an old man. He then developed tremulousness, clumsiness in movements and sensitivity to cold. The hair of the pubis and chest grew more rapidly while the hair of the scalp fell out. On a visit to his home his parents were startled by his changed appearance.

He was admitted to another hospital where on physical examination he was described as having a ruddy, puffy face; a "bull neck"; striae of the buttocks, thighs and right axilla and obesity of the abdomen. The musculature was flabby. The remainder of the examination was normal and the blood pressure was 110 systolic and 86 diastolic. Laboratory studies revealed a basal metabolic rate of minus 29 per cent, diminished glucose tolerance, and generalized diffuse osteoporosis of the vertebrae. The patient was transferred to Walter Reed General Hospital.

Physical examination. On admission, the temperature, pulse rate and respiratory rate were normal, the blood pressure was 138 systolic, 102 diastolic, his weight was 137 pounds and his height 65½ inches. His face was plethoric with heavy jowls, and the eyes were prominent. There was a fat pad over the seventh cervical vertebra, and marked purplish striae were seen in the axillae and over the buttocks and thighs. There was a feminine distribution of pubic hair and of body fat, and the breasts were slightly enlarged. The abdomen was pendulous and the right kidney questionably palpable. The testes and penis were smaller than normal, and the cremasteric reflex was absent. All muscles had poor tone.

Laboratory data. The erythrocyte counts and hemoglobin determinations were within normal limits. The leukocyte counts varied from 9100 to 10,000 with 71 to 88 per cent polymorphonuclears. The blood sugar was 93 mg., cholesterol 362 mg., urea nitrogen 6 mg., chlorides 430 mg., phosphorus 3.3 mg., calcium 10 mg., alkaline phosphatase 2.0 Bodansky Units and vitamin A 64 International Units (normal 65-165), all values being per 100 ml. of blood. The

* From the weekly Clinicopathologic Conferences, Walter Reed General Hospital, directed by V. H. Cornell, Colonel, MC. Received for publication, May 6, 1948.

glucose tolerance test showed the following values: fasting level, 72 mg.; one-half hour, 168 mg.; two hours, 133 mg.; three hours, 81 mg. Urinalyses were consistently negative. Basal metabolic rate was minus 30 per cent. X-ray films of the skull revealed the sella turcica to be small and over-bridged, films of the kidneys and urinary bladder were negative, and intravenous urograms were normal. Injection of the right perirenal region with air revealed no evidence of tumor. The electro-encephalogram was normal. Four weeks after admission an operation was performed.

CLINICAL DISCUSSION

Ezra M. Greenspan, Captain, M.C. "This interesting patient seems to have been the victim of a process affecting his carbohydrate metabolism, salt and water balance, skin and subcutaneous tissues and secondary sexual characteristics. Of the various hormones of the endocrine glands, only those of the adrenal are capable of producing the marked changes observed in this patient. We are, therefore, safe in assuming that he had some abnormality of the adrenal glands. It is also probably safe to assume that the changes noted were due to overactivity or hypersecretion of some or all of the various hormones produced by the adrenal cortex. Confirmation could have been obtained by means of a determination of the 17-ketosteroids. Under certain circumstances fractionation of the 17-ketosteroids into alpha and beta alcoholic fractions, as well as non-alcoholic fractions, may help in a pathologic differentiation of hyperadrenalism. From 85 to 95 per cent of the normal ketosteroid excretion is in the form of the alpha alcoholic fraction; however, in adrenal carcinoma and in cases of adrenal hyperplasia there is usually a marked increase in the beta alcoholic and non-alcoholic 17-ketosteroids, as well as, of course, a total increase in the 17-ketosteroid output.

"The clinical diagnosis of Cushing's syndrome is suggested by the following findings in this patient: buffalo type of adiposity, moon face, bull-neck, hypertrichosis, purple striae, plethora and cutis marmorata. The patient, apparently, did not have acrocyanosis. It is also suggested by the presence of edema of the extremities and hypertension as well as polyuria. Since his clinical disease was of less than one year's duration, the hypertension was mild, and there was no evidence of hypertensive cardiovascular disease or nephritis. These may not only be the findings characteristic of early Cushing's syndrome, but also may reflect the production of hormones affecting primarily the carbohydrate metabolism and the sexual characteristics rather than the salt and water balance. The patient did not have erythremia, but he did have neutrophilia. These are frequently observed in more advanced states of Cushing's syndrome. The kyphosis and the diffuse osteoporosis were apparently quite conspicuous among the clinical findings. Albright¹ is of the opinion that these are manifestations consistent with the markedly negative nitrogen balance in hyperadrenalism. His theory is supported by the observation that treatment with testosterone may result in a marked improvement in osteoporosis and the development of a positive nitrogen balance. The abnormal glucose tolerance test revealed the

presence of impaired carbohydrate metabolism. This is presumably due to excessive production of the so-called "S" hormones^{1,3} which are believed to cause excessive production and excretion of carbohydrates by the uncontrolled conversion of body proteins in the processes of gluconeogenesis. In these patients there is usually a tendency towards diabetes mellitus, and, in fact, at least one case is on record of hyperadrenalism manifested solely by diabetes mellitus.¹¹ Insulin resistance is usually present, together with a marked impairment in the accumulation of glycogen reserve. Our patient showed clinical evidence of muscle atrophy and weakness. These are also a reflection of the markedly negative nitrogen balance in this syndrome and may be favorably affected by the administration of testosterone. The urea nitrogen of 6 mg. per 100 ml., if correct, probably reflects some measure of hepatic insufficiency. The hypercholesterolemia of 362 mg. per 100 ml. is not uncommon in Cushing's syndrome. It is a manifestation of morbid fat metabolism and suggests fatty infiltration of the liver such as may be seen in untreated diabetes mellitus. Somewhat atypical is the basal metabolic rate in this patient. It is well known that the basal metabolic rate in Cushing's syndrome may be high, or normal and occasionally low due to changes in metabolism, as well as to the alterations in body weight, body configuration and surface area. Consequently, the basal metabolic rate is somewhat unreliable in a characterization of Cushing's syndrome. One could, of course, speculate and say that in patients with marked increments in body fat the lowered metabolism of fat tissue accounts for hypometabolism as is seen in this case. Certain other clinical features deserve comment, exophthalmos is frequently observed in Cushing's syndrome, dull frontal headaches are also very common, and lastly, the tendency toward feminization in this patient is not to be considered unusual.

"The pathologic findings in Cushing's syndrome have been the subject of great controversy since the original description of Cushing in 1932. Cushing observed marked changes in the basophilic cells of the pituitary gland, but in only a very few of his cases were there any definite adenomata of the pituitary. Since then, it has been learned that true basophilic adenoma formation with Cushing's syndrome is seen in fewer than 10 per cent of patients in any series. Although Cushing noted certain changes in the adrenal glands in his patients, it was his opinion that these were secondary to abnormal pituitary function. More recently, numerous observers have reported cases of adrenal carcinoma with the clinical manifestations of Cushing's syndrome. Albright¹ and his collaborators³ claimed that a still more common pathologic finding is bilateral adrenal hyperplasia of a type frequently recognizable only by microscopic studies. The occurrence of arrhenoblastoma and thymus gland tumors has occasionally been associated with Cushing's syndrome. In any event the pathologic findings in cases of Cushing's syndrome frequently produce much argument because of the limitations of present cytochemical methods of study.

"In our patient, the sella turcica was said to be small and overbridged. This is perfectly consistent with Cushing's syndrome since neither expansion nor erosion of the sella turcica occurs from basophilic adenoma. The right perirenal

insufflation was thought to rule out any fairly large tumor; I presume it was performed upon the right side because one observer reported enlargement of the right kidney. The intravenous pyelogram was normal which also suggested that no large tumor was present in the region of either adrenal. There was no calcification in the region of the adrenal glands; this occurs occasionally in the presence of adrenal carcinoma. Nor was there any evidence of a thymus gland tumor in this patient.

"The operation probably consisted of a bilateral adrenal exploration. If adrenal carcinoma is found on one side, the other adrenal will probably be atrophic. If either a normal or an atrophic gland is found on one side, there is no assurance that the condition of the gland on the opposite side is either normal or diseased. In any event, the most careful and meticulous exploratory technic is necessary, and the patient must be prepared for treatment of an acute postoperative Addisonian crisis which may follow after procedures of this kind. The prognosis with this type of surgery is very poor, even with the use of massive amounts of a cortical extract and synthetic hormone. If a patient with adrenal carcinoma succumbs to postoperative acute adrenal insufficiency, it is because our replacement therapy is still far from complete. Perhaps the newer synthetic hormones of the 17-hydroxy-type will provide us with a valuable therapeutic aid when they become commercially available. Patients with bilateral hyperplasia may have a very stormy course postoperatively if too much manipulation has occurred to the glands during the surgical exploration. Resection of a portion of both adrenal glands is always hazardous since the remaining tissue may become devitalized. On the other hand unilateral partial resection in the presence of adrenal hyperplasia is also unsatisfactory. Roentgen therapy and testosterone occasionally produce temporary clinical remissions, but, more often, the patient deteriorates slowly during the remainder of his life, the expectancy of which is approximately five years. Death usually occurs from cardiovascular or renal complications."

DIAGNOSES

Clinical: Cushing's syndrome due to bilateral adrenal hyperplasia.

Captain Greenspan: Cushing's syndrome, probably due to adrenal hyperplasia.

Anatomic: Cushing's syndrome due to bilateral adrenal hyperplasia; postoperative acute adrenal insufficiency due to infarction of right adrenal.

PATHOLOGIC FINDINGS AND DISCUSSION

Lorenz E. Zimmerman, Captain, M.C. "The operation performed consisted of a bilateral exploration of the adrenals, which necessitated resection of both twelfth ribs. The left adrenal appeared normal, but there was some accessory adrenal tissue behind the upper pole of the kidney. This was entirely resected together with part of the normal-appearing adrenal. The adrenal on the right side was considered to be small, and again accessory adrenal tissue was found in the perinephric fat. This and part of the main gland were resected. Specimens received in the laboratory consisted of 1 Gm. of left adrenal tissue and 0.5

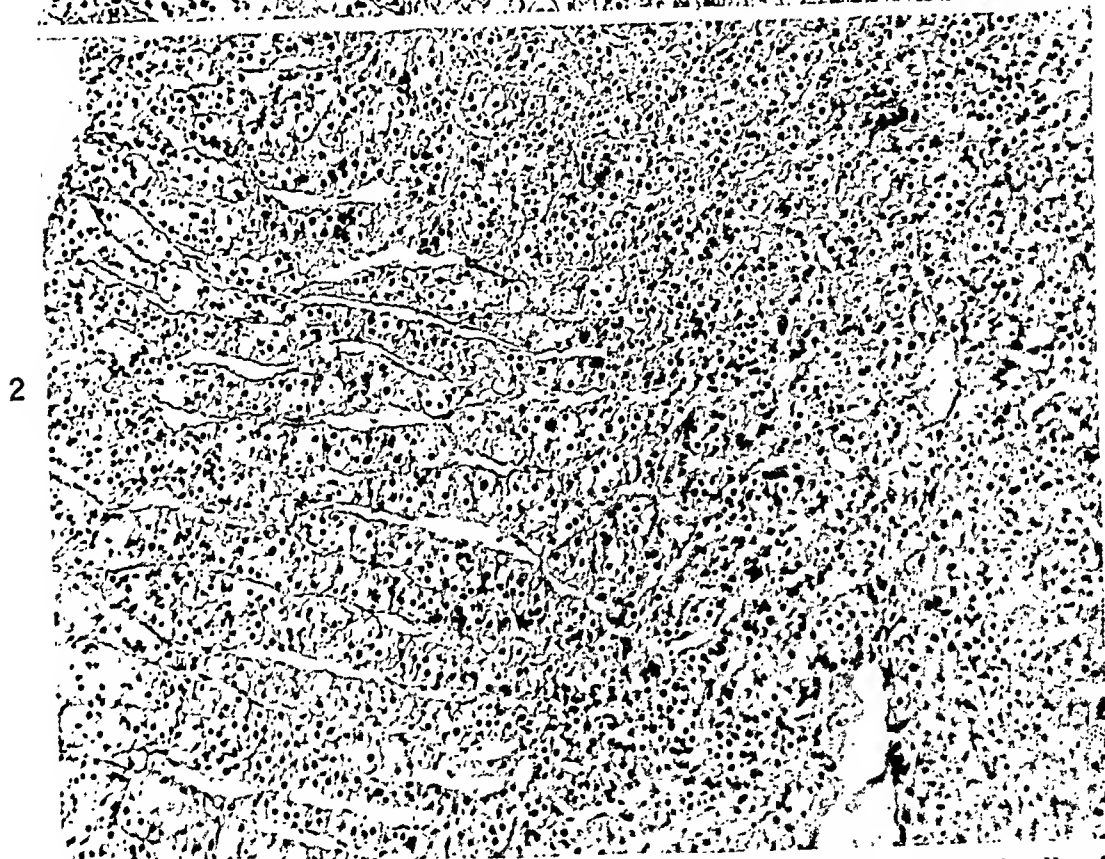


FIG. 1. From surgical specimen of adrenal showing high lipid content of cells of zona glomerulosa and fasciculata.

FIG. 2. From surgical specimen of adrenal showing acidophilic cells of reticular zone.

Gm. of right adrenal tissue.. Microscopically, the tissue revealed moderate cortical hyperplasia without adenoma formation. The peripheral cells were characterized by a high content of lipoidal material (Fig. 1) while the reticular zone contained deep acidophilic cells (Fig. 2).

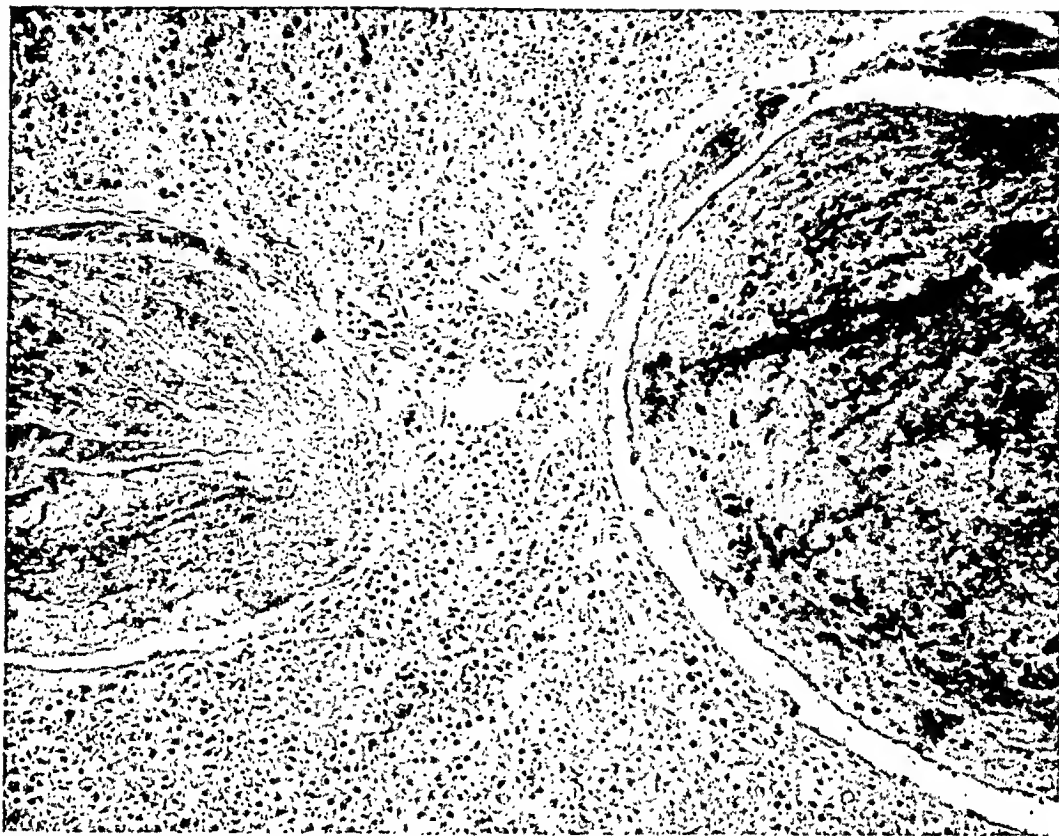
"Postoperatively, the patient did well for twenty-four hours, being treated with saline infusions, adrenal cortical extract and adrenalin. The next day he suddenly went into profound shock, but was revived by intensive therapy with cortical extract, plasma and saline intravenously, and oxygen. After temporary improvement, his condition again became critical, and there was marked cyanosis. Therapy was of no avail, and the patient expired about forty-eight hours following the operation.

"Autopsy was performed two hours after death. The external findings were essentially those noted on physical examinations during life. It was noted on removing the surgical dressings that the epidermis was so atrophic that it tended to peel off with the adhesive tape. The organs of chief interest were the adrenals, pituitary, liver and thyroid. Microscopically, the right adrenal showed in addition to the hyperplasia described in the surgical specimens, extensive infarction due to thrombosis of the central vein (Figs. 3 and 4). Crooke's hyaline changes⁵ were seen in almost all the pituitary basophils (Fig. 5), but there was no adenoma. The liver was the seat of severe central fatty metamorphosis, only a peripheral zone of the hepatic lobules remaining (Fig. 6). The thyroid showed evidence of inactivity with large colloid-filled follicles and thin, flattened lining cells.

"Captain Greenspan has discussed the controversial pathologic basis of Cushing's syndrome. Most authorities today agree that the adrenal changes are most important. Albright¹ states that in every case some adrenal cortical change is present, usually bilateral hyperplasia, less often adenoma or carcinoma. Goldzieher⁷ described two histologic changes in cortical hyperplasia with Cushing's syndrome. One is the storage of lipoids in the outer layers, but this is not specific as he observed it in other cases without signs or symptoms of Cushing's syndrome. The most important change is the formation of an unusually broad reticular layer which stains intensely with eosin. This finding was not observed on microscopic examination of thousands of adrenals from patients with other conditions and is, therefore, considered specific for Cushing's syndrome. In this connection it is of interest that Vines, working with Broster,⁴ was able to demonstrate fuchsinophilic granules in the reticular layer only in the adrenals of women showing virilism. Recently, Pedersen and Kenyon⁹ reported a case of Cushing's syndrome with virilism in which the adrenals at operation were grossly normal, as was the biopsy specimen, except for the presence of fuchsinophilic granules.

"Albright¹ and his colleagues³ have clarified the bewildering pathologic physiology of Cushing's syndrome by introducing the concept of two main groups of adrenal cortical hormones, the "N" and "S" hormones. Since these have essentially opposite pharmacologic effects, the syndromes produced by their excessive secretion will tend to be opposite. Thus, in the adrenogenital syndrome there is excessive production of "N" hormones which stimulate somatic and

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FIG. 3. From autopsy specimen of adrenal showing thrombi in central vein.

FIG. 4. Edge of adrenal infarct showing border of inflammatory reaction.

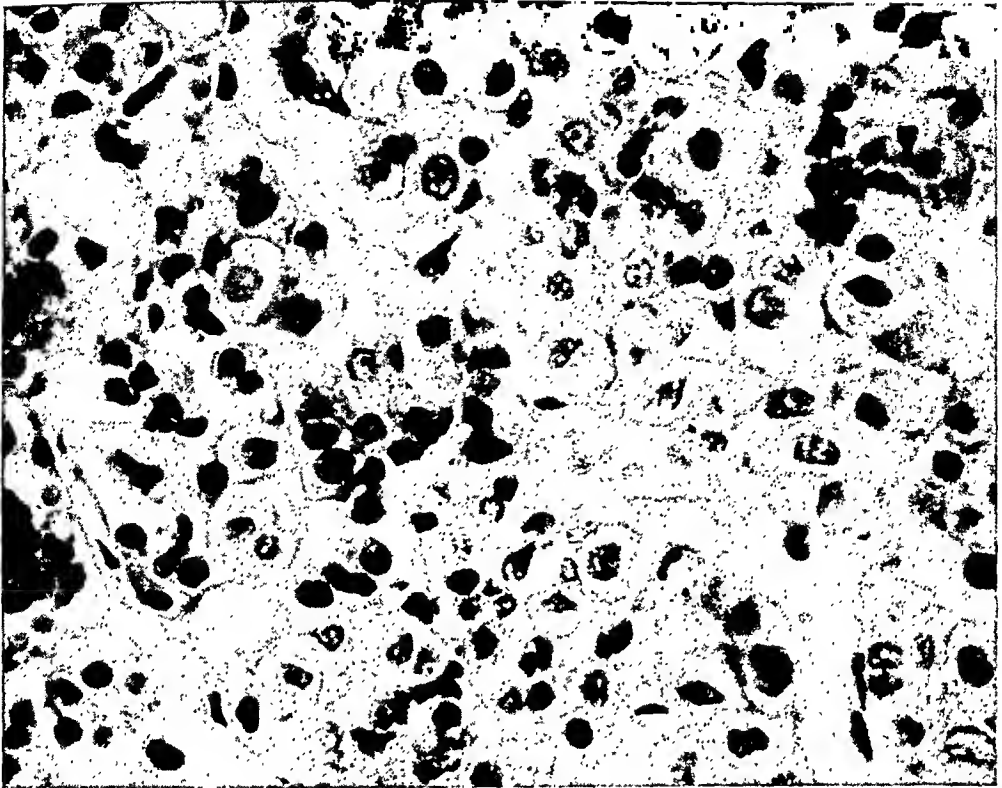


FIG. 5. Extensive Crooke changes in pituitary. Basophils are swollen with vacuolated acidophilic cytoplasm.

FIG. 6. Fatty metamorphosis of liver. Only the cells at the periphery do not show the extensive change.

sexual development, while in Cushing's syndrome the "S" hormones, which inhibit anabolism, predominate. This leads to atrophic skin and muscles, osteoporosis, capillary fragility, amenorrhea and impotency. In each of these syndromes, however, there is a compensatory increased production of hormones opposite to that being liberated by the cortical tumor or hyperplasia. This naturally leads to considerable variations and mixtures of the two syndromes and explains why these syndromes are usually not seen in pure form.

"The operative results in this case emphasize the great hazard of bilateral partial resection in cases of cortical hyperplasia. Most authorities agree that the procedure of choice is left total adrenalectomy.^{4, 7, 8, 10} This is a relatively safe operation, and the results obtained are generally satisfactory. Walters' experience,¹² however, was disappointing, but he observed good results from pituitary irradiation."

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SELECTED ABSTRACTS

The Genus Shigella and Shigellosis. E. NETER. Am. J. Digest. Dis., 15 (7): 213-232, 1948.

This is a very comprehensive review of the *Shigella* problem, with 195 references and several tables showing the differences between the various strains and their nomenclature. Neter suggests some modifications of the classification of *Sh. paradyserteriae*, with regard to the newly described types VII and VIII of Francis, *Sh. ctousae* of Lavington and the so-called Sachs and Wheeler-Stuart types. He considers *Sh. alkalescens* as pathogenic but withholds final comment on *Sh. dispar*.

S.S. agar, D.C. medium, MacConkey's plate, selenite-F broth and the enrichment fluid of Bangxang and Eliot are recommended for the isolation of shigellae with emphasis on the fact that the highest percentage of successful isolations is achieved by the simultaneous use of several mediums. The colonies are transplanted to triple sugar iron agar, then seeded to a set of biochemical (carbohydrate) tubes and tested with diagnostic serums.

The problem of active immunization has not been solved as yet. Chemoprophylaxis and chemotherapy yielded, however, satisfactory results, permitting a rather effective control of shigellosis.

Chicago

O. FELSENFELD

Experimental Enterococcal Food Poisoning in Man. A. G. OSLER, L. BUCHBINDER AND G. I. STEFFEN. Proc. Soc. Exper. Biol. and Med., 67 (3): 456-459, 1948.

The authors described previously several outbreaks of mild food poisoning (Pub. Health Rep., 63: 109, 1948) in which enterococci were the predominant organisms isolated from the suspected food. In order to determine if enterococci may cause food poisoning, 21 volunteers were fed with twenty-hour old cultures. Clinical symptoms did not develop. Another group of volunteers was fed with egg salad, custard and sterile milk that had been inoculated with enterococci and incubated for five hours. Six volunteers developed symptoms (nausea, vomiting and/or diarrhea) of mild food poisoning.

O. FELSENFELD

Studies in Acute Diarrheal Diseases. XXX. Salmonellosis in Florida. M. M. GALTON AND A. V. HARDY. Pub. Health Rep., 63: 847-851, 1948.

During the years from 1942 through 1946, 81,174 stools, mostly from foodhandlers, were examined. Kauffmann's combined enrichment and plating method was found to be most efficacious. *S. typhosa* was isolated from 510 specimens, and other *Salmonella* strains belonging to 48 different types, were isolated 748 times. Seven new types were diagnosed and no *S. paratyphi A* was found. The commonest salmonellas isolated in order of frequency were: *S. anatum*, *S. derby*, *S. newport*, *S. oranienburg*, *S. typhi-murium*, *S. miami* and *S. montevideo*. The carrier state was often transient.

O. FELSENFELD

Salmonella Typing in Ontario and the Use of Polyvalent Antisera. V. CROSSLEY, M. FERGUSON, P. IRVINE AND M. HASTINGS. Canad. J. Pub. Health, 39 (5): 192-199, 1948.

Twenty-seven types of *Salmonella* were identified. *S. tennessee*, *S. anatum*, *S. reading* and *S. bonariensis* were of animal origin; *S. enteritidis*, *S. montevideo*, *S. choleraesuis*, *S. typhi-murium*, *S. paratyphi B* and *S. oranienburg* from both man and animal; the remaining types were all of human origin. Altogether 1,441 strains were classified. *S. typhosa*, *S. typhi-murium*, *S. paratyphi B* and *S. newport* were the most frequent types. Four pools of "O" and four pools of "H" serums were used in the classification of the strains. These serums were prepared according to a modified procedure of Kauffmann.

O. FELSENFELD

Enterococci in Urinary Tract Infections. G. GIERTZ. Acta chir. Scandinav., 94: Suppl. 109, 1-168, 1946.

Of 273 streptococci isolated from the urine, 2 were microaerophilic. There were 240 strains of enterococci, 97 of the main type; 45 of the variety liquefaciens, 93 of the variety hemolyticus, and 5 of the variety zymogenes. A pH of the urine slightly above 5 favored the growth of most enterococci. None of the strains split urea to any considerable extent. Sulfathiazole in a concentration of 180 mg. per 100 ml. had no effect or only a feeble effect on enterococci. While potassium permanganate, boric acid and silver nitrate had an irregular effect on these microorganisms, chloramine was very effective. Enterococci were never found in normal urine. They were most frequently encountered in urologic cases after instrumentation. Enterococci were detected in 85 per cent of the positive cultures of urine. They were, however, often difficult to distinguish from staphylococci.

O. FELSENFELD

Estudio de Cien Casos de Salmonelosis Humanas. (Study of One Hundred Cases of Human Salmonellosis.) G. VARELA, J. LAGUNA AND J. ZOZAYA. Rev. d. Inst. salub. y enferm. trop., 8 (1): 15-28, 1947.

Salmonellae were isolated from 60 children and 40 adults in Mexico. The clinical picture in children and adults, respectively, was the following: simple diarrhea in 21.6 and 57.5 per cent, choleric form syndrome in 23.3 and 28.5 per cent, dysenteric form symptoms in 15 and 7.5 per cent, and mixed type in 18 and 7.5 per cent of the cases. The average duration of illness was 7.35 and 5.58 days, respectively. *S. typhimurium*, *S. derby* and *S. newington* were the most frequent strains. Twenty-nine types were isolated. Blood cultures were positive in 32.8 and bile cultures in 18 per cent of the cases. Proctoscopic examination revealed no changes in 57, diffuse congestion of the mucosa in 13, induration of lymph nodes in 15 and mucosal defects in 19 of the patients. Only 6.8 per cent eliminated salmonellae for more than 90 days. No adults died but 14 children succumbed to salmonellosis. Of the complications, otitis media occurred 6 times, tonsillitis 3 times and bronchopneumonia twice.

O. FELSENFELD

Infecciones Entericas por Shigelas en Casos de "Diarreas Infantiles de Verano". (Enteric Infections with Shigellas in Cases of "Infantile Summer Diarrhea".) J. J. MONTEVERDE AND C. DE SIMONE. Arch. farm. y bioquím. Tucumán, 3 (3): 309-326, 1947.

Out of 239 cases of so-called infantile summer diarrhea, 36 (about 15 per cent) harbored shigellae. Seventeen strains belonged to the *Sh. paradysenteriae* group (types I, II, III, I-III and VIII), 13 were *Sh. sonnei*, 4 *Sh. alkalescens* and 2 *Sh. ambigua*. Among 116 healthy persons two were found to carry *Sh. paradysenteriae*. The study was performed in Buenos Aires during the years 1945 to 1947.

O. FELSENFELD

Estudio de Una Nueva Fiebre Petequial Aislada en Michoacan (República Mexicana) del Rhipicephalus sanguineus. (A New Petechial Fever Isolated in Michoacan (Mexico) from Rhipicephalus sanguineus.) M. E. BUSTAMANTE, G. VARELA AND E. ROCH. Rev. d. Inst. salub. y enferm. trop., 8 (5): 163-174, 1947.

Triturated material from the tick *Rhipicephalus sanguineus* collected in Michoacan produced in guinea pigs a disease which resembled Rocky Mountain spotted fever clinically but differed from it serologically. No cross-immunity could be produced with two typhus fever and two Rocky Mountain spotted fever strains. Complement-fixation tests with endemic and epidemic typhus, spotted fever and rickettsialpox antigens were also negative. Rickettsias were isolated from the tunica vaginalis of the inoculated guinea pigs and successfully cultured in the yolk sack of developing chick embryos. "Michoacan fever" is suggested as the name of this new rickettsial disease.

O. FELSENFELD

Cholera in Egypt. M. A. GOHAR AND M. MAKKAWI. *J. Trop. Med.*, 51 (5): 95-99, 1948.

A recent cholera outbreak started in the village of Korein. Its source could not be detected. The causative agent was the Inaba type of the vibrio. The epidemic spread rapidly through the southern provinces of Cairo, chiefly along the Nile valley but did not invade large cities. Date fruits are believed to have played the principal role in the propagation of the infection. The epidemic was brought under control in two and one-half months.

The usual routine of inoculating stools into alkaline peptone water was not always successful because certain strains of *Alcaligenes faecalis* proved to be just as rapid in their surface growth as the cholera vibrios. Some of these strains even were agglutinated with anti-cholera serums. Potassium tellurite (1:50,000) checked many of the contaminating organisms. Convalescent carriers and contact carriers did not harbor vibrios for more than ten days.

General vaccination of the entire population with one dose of 8,000 million heat-killed vibrios was carried out.

The strains isolated from the epidemic survived in crude Nile water for nine days, on vegetables and linen for five days.

O. FELSENFELD

Arteriosclerosis in a Nine Year Old Child. J. L. GEDGOUD AND J. P. TOLLMAN. *Lancet*, n. s., 68: 197-198, 1948.

The authors report a case of moderately severe arteriosclerosis which occurred in a nine year old girl. The child complained of marked respiratory distress. The blood pressure was 218/176. Urinalyses showed three-plus albumin, specific gravity ranging from 1.015 to 1.028, and white blood cells but no red blood cells. The child continued to be orthopneic and died in cardiac failure. At necropsy, the arterioles of the kidneys, pectoral muscles and spleen showed marked thickening of their walls.

The authors considered that the etiology of the hypertension in this case was possibly associated with a previous pyclo nephritis.

Cleveland

BEN FISHER

Serum and Plasma Antithrombin. CHARLES A. OWEN, JR. *Proc. Soc. Exper. Biol. and Med.*, 67: 367-369, 1948.

Blood has the ability to inactivate added thrombin, as well as spontaneously formed thrombin. The author suggests a method of estimating the rate of natural antithrombin activity. By this method, dog plasma seemed to exert about the same antithrombin activity regardless of the prothrombin level after dicumarolization. The antithrombin of dog serum varied inversely with the prothrombin content of the original plasma.

Owen suggests that antithrombin activity be determined on plasma, unless due consideration is given to the antithrombin activity already performed by the serum.

BEN FISHER

Enhancing Effect of Mg^{++} on Clotting Activity of Ca^{++} . FRANK MALTANER. *Proc. Soc. Exper. Biol. and Med.*, 67: 301-303, 1948.

The action of ionized calcium salts in the blood coagulation process has been shown to be highly specific since the salts of even closely related elements, strontium and barium, have relatively little effect.

Ionized magnesium salts ($MgCl_2$) alone do not possess the coagulative activity of calcium salts. However, they do enhance the effect of calcium. This is more apparent when the calcium concentration is relatively low, and the concentration of magnesium ions is greater than that of calcium. Magnesium ions have been previously shown to enhance the complementary activity of blood. The author, therefore, feels that this parallelism between enhancing effects indicates the close relationship between complement and blood coagulation.

BEN FISHER

Prothrombin Activity of Stored Human Blood. FRED J. SCHILLING, ALBERT DENATALE, AND LUIS A. AMILL. *Am. J. Med. Sc.*, 215: 415-418, 1948.

Previous *in vitro* studies on stored (banked) human blood have shown it to be deficient in prothrombin. The authors studied fifty different samples of stored human blood and found it to be adequate in prothrombin activity for the first five to seven days. This is considered to be significant in the replacement of whole blood following the hemorrhage of Dicumarol-induced prothrombinopenia, or of the hypoprothrombinemia of severe liver damage.

The prothrombin activity in the bloods studied was 100 per cent (by the Link-Shapiro technic) for the first five days of storage; there was a gradual drop to 84.8 per cent by the ninth day, followed by a more rapid drop to 66.2 per cent on the twelfth day. The depression continued slowly to 47.7 per cent by the twenty-first day. For this study, 500 cc. of blood was drawn from healthy donors and mixed with 125 cc. of acid-citrate-dextrose solution, as commonly employed in many blood banks.

BEN FISHER

Clinical and Laboratory Studies on the Uptake of Radioactive Phosphorus by Lesions of the Breast. H. J. McCORKLE, B.V.A. LOW-BEER, H. GLENN BELL AND ROBERT S. STONE. *Surgery*, 24: 409-415, 1948.

Tracer doses of radioactive phosphorus (P^{32} incorporated into Na_2HPO_4) were given intravenously to patients with breast lesions. After forty-eight hours, skin surface measurements of radioactivity were done over the lesion and over a comparable area on the opposite side. Increased metabolic activity results in rapid uptake of phosphorus, and is expressed in an elevation of the surface radioactivity over the lesion as compared with the values found in comparable unaffected areas of the same patient. An increase of more than 25 per cent was considered significant by the authors.

Twenty-one patients with benign breast lesions were examined. Of 17 patients with fibrocystic disease or benign tumors, an elevated reading was obtained only in one person who had fibrocystic disease. In 4 patients with benign inflammatory disease, increased surface radioactivity was found in all, ranging from 35 to 392 per cent.

Study of 41 patients with mammary carcinoma or with lymph nodal metastases demonstrated in 34 instances elevated radioactivity over the tumors; in the various groups the increase averaged from 40 to 140 per cent. Tracer studies failed to indicate elevation of the surface radioactivity over the tumors in 3 patients with mucoid carcinomas, in two deeply situated carcinomas, in one carcinoma demonstrable only microscopically, and in one following radiation and hormone therapy. High cellularity, and ulceration or infection of the tumor appeared to favor the uptake of radiophosphorus.

Chicago

KURT STERN

BOOK REVIEWS

Studies of the Renal Circulation. By JOSEF TRUETA, M. D., HON. D. SC., (OXON.); ALFRED E. BARCLAY, O.B.E., D.M., F.R.C.P., F.F.R., F.A.C.R.; PETER M. DANIEL, M.A., M.B.; KENNETH J. FRANKLIN, D.M., F.R.C.P.; AND MARJORIE M.L. PRICHARD, M.A.; from the Nuffield Institute for Medical Research, Oxford. 187 pp., 83 figs. \$10.00. Springfield, Ill.: Charles C Thomas, 1947.

Studies of the Renal Circulation is a fascinating story of the search by Trueta and his collaborators for an explanation of the fatal anuria which was observed to occur in air-raided victims suffering crushed extremities. A variety of methods of attack were brought to bear on the problem by this team of specialists and, as a result, a new concept of the renal circulation has been formulated. In short, it was demonstrated that compression of an appendage causes a reflex constriction of the femoral and renal arteries, the narrowing of the renal vessel being dependent upon the integrity of the splanchnic nerves. Furthermore, under such circumstances the blood is shunted through the medulla of the kidney by way of the vasa recta thus creating an ischemia of the cortex and impairing its urine-producing function. A similar rerouting of blood through the kidneys was induced by several different procedures, including the injection of staphylococcus toxin or drugs, nervous stimulation and hemorrhage.

These studies represent a milestone in the history of renal research. One has only to read the chapter on Pathological and Clinical Implications to realize that the information presented affects every aspect of our knowledge of renal function and serves as the basis for a rational explanation of many previous clinical and experimental observations. Criticisms could be raised against the book but these are of minor significance when viewed in the light of the importance of the work as a whole. *Studies of the Renal Circulation* is essential reading for the scientist who wishes to deal intelligently with any clinical or experimental problem pertaining to the kidneys.

Ann Arbor, Michigan

BURTON L. BAKER

The Salicylates, A Critical Bibliographic Review. By MARTIN GROSS, M.D., Research Assistant (Assistant Professor) Laboratory of Applied Physiology, Yale University; and LEON A. GREENBERG, PH.D., Associate Professor, Applied Physiology, Yale University. With an introduction by Howard W. Haggard, M.D., Director, Laboratory of Applied Physiology, Yale University. 380 pp., 20 figs., 29 tables. \$6.00. New Haven: Hillhouse Press, 1948.

This, the second in a series of bibliographic reviews emanating from the Institute for the Study of Analgesic and Sedative Drugs at Yale University, fully maintains the excellent features of the first volume on acetanilid. The extensive employment of salicylate compounds in disease and as an analgesic has resulted in an enormous amount of scientific literature of widely diversified nature. The authors, Dr. Gross and Dr. Greenberg, have admirably succeeded in incorporating nearly all of these references, totally more than 4000, into a thorough, succinct and highly critical review of all phases of the subject. The book is divided into approximately two equal parts. The first part contains the text which is divided into seven chapters as follows: 1. historical; 2. occurrence and properties of salicylates; 3. fate of salicylates in body; 4. pharmacology and toxicology of salicylates; 5. therapeutic uses; 6. salicylate poisoning; 7. question of addiction or habituation. The second part is a bibliography of 4,093 references. In each chapter most of these references are cited in the text. At the end of each chapter there are additional references on each of the subjects discussed.

The clinical pathologist will find much of interest in this book. Probably, the portions of most immediate value are the sections on salicylate intoxication. This reviewer has nothing but the heartiest commendations to the authors on their painstaking and critical review of the field.

Boston

WALTER W. JETTER

Il Principio Antianemico—Perniciosa, Natura, Meccanismo D'Azione, Dosaggio. By G. ASTALDI AND M. BALDINI. Prefazione by Paolo Introzzi. Estratto De Il Farmaco, Scienza and Tecnica. 234 pp. lire 1200. Pavia: Premiata Tipografia Succ. Frat. Fusi, 1948.

This work by Astaldi and Baldini is the first attempt to bring together in one volume the most important contributions on the nature, mechanism and dosage, and the pharmacologic, chemical and biologic facts concerning the anti-pernicious anemia principle.

In the first part of the monograph, which deals with the nature and diffusion of the hemopoietic principle, the authors review the work of Whipple and his associates, Minot and Murphy, Castle and others. There is a discussion of the specific effect of liver extract, gastric extract and folic acid on the blood and bone marrow in pernicious anemia. The second part of the monograph is devoted to an extensive study of the evaluation of the methods used in assaying and determining the dosage of liver extract. There is a valuable presentation on the limitation of the reticulocyte crisis as used to determine the potency of the hemopoietic principle. The authors emphasize that the reticulocyte crisis observed in pernicious anemia is a biologic phenomenon, very labile and sensitive to the influence of many factors, which can only be realized if the conditions are those of a perfect experiment. Of particular interest is the chapter on experimental anemia in relationship to the anti-pernicious anemia principle. From the experimental work on bone marrow cultures the authors conclude that the method of Race and Fischer (migratory cellular phenomenon) for evaluating the activity of anti-pernicious anemia liver extract preparations lacks specificity. In the final chapter Astaldi and Baldini present a new test for evaluating the potency of the anti-pernicious anemia principle in liver preparations based on an erythroblastometric reduction and successive maturation phases of the erythroid cells as observed following serial sternal marrow aspirations.

This monograph is a valuable and authoritative contribution to the subject of pernicious anemia.

Chicago

LOUIS R. LIMARZI

I Veleni della Mitosi. I Fattori che Influiscono Sull'Attività Reproduttiva delle Cellule con Particolare Riguardo ai Reperti Ottenuti su Culture in Vitro. By G. ASTALDI, C. MAURI AND A. ALLERGI, Estratto De Il Farmaco, Scienza and Tecnica. 105 pp. lire 700. Pavia: Premiata Tipografia Succ. Frat. Fusi, 1947.

The monograph offers a wealth of information on the mechanism of cell division under normal and pathologic conditions and the modification of this mechanism following the use of certain chemicals, drugs and physical agents.

The work is divided into three parts. The first part deals with the rhythm and a measure of the several phases of cell division and interkinesis (resting stage) with a study of the dependence and effects of temperature on this mechanism. The second portion of the monograph is a study of the influence of the medium on the reproductive activity of cell division. The remaining chapters which comprise the greater part of the book are a comprehensive review with experimental data on the effect of certain chemicals, drugs and physical agents on the mechanism of cell division under normal and pathologic states. These include colchicine, sulfanilamide, penicillin, ultra-violet light and roentgen ray. The bone marrow culture methods used in some of these studies are those previously described by these authors and Feisch.

This monograph represents a valuable contribution to the changes in cell morphology and karyokinesis following injury or poisoning in normal and pathologic states.

LOUIS R. LIMARZI

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of the

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Volume 18

MUNCIE, INDIANA

1948

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